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Effect of starter nutrition on compensatory growth and development of swine

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INTRODUCTION

In the past years, much attention has been given to the effect of early nutrition on the subsequent performance and body composition of domestic animals. Unlike rats, pigs and other farm animals are able to recuperate in their growth after periods of feed or nutrient restriction. If a normal diet is fed generously after the restriction is removed, the normal body size and composition of these farm animals are usually attained. This recuperative performance is termed as compensatory growth (Bohman, 1955). On the other hand, rats which were subjected to either undernutrition or malnutrition (deficiency in one of more nutrients) during early life showed growth stunt; poor feed and protein utilization; kidney malfunction; lower than normal concentration of protein, ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) in various tissues and organs; lower than normal total cell number, adipose cell number in epididymal fat pad; and abnormal body conformation, regardless of how well they were subsequently fed.

The purpose of this study was to determine the effect of variations in early nutrition on cellular response, on efficiency of protein utilization, on plasma metabolite patterns during various parts of recovery period and on body composition of pigs at the end of treatment and recovery periods.

LITERATURE REVIEW

Compensatory Growth

History

Compensatory growth is growth which proceeds at enhanced rates during realimentation following a period of restricted nutrition or malnutrition, with eventual normal body size and conformation being achieved (Bohman, 1955). Compensatory growth has long been observed in beef steers (Waters, 1908), in human (Stearns and Moore, 1931), in pigs (McMeekan, 1940), in lambs (Palsson and Verges, 1952) and in poultry (Wilson, 1952). In their reviews, Palsson (1955) and Wilson and Osbourn (1960) pointed out that after the growth restriction had been removed, farm animals had a remarkable capacity to grow and to maintain the constancy of homeostasis in their bodies. Clarke and Smith (1938) observed that rats which were 50 percent restricted-fed for three weeks subsequently gained weight so rapidly that by the end of nine weeks their weights exceeded those of the controls. This phenomenon, which was termed "over-compensation", was pointed out by Wilson and Osbourn (1960) to be associated with an excess deposition of fat in the body. Compensatory growth, according to Pomeroy (1955), is partly due to a replacement of fat in adipose tissues which have been depleted during the restriction period. Ragsdale (1934) has suggested that undernutrition disturbs the normal relationship between chronological age and physiological age in such a way that, in the restricted-fed animal, physiological aging proceeds at a slower rate. When such an animal is realimentated, it

tends to grow at a rate appropriate to its physiological age rather than to its chronological age (Winchester and Ellis, 1957).

Nutrition and compensatory growth in pigs

Since the time of McMeekan (1940) much work has been done with particular emphasis on the effect of early nutrition on subsequent performance and carcass quality or chemical composition of market pigs. Compensatory growth has been consistently shown in pigs fed ad libitum after a period of feed restriction to about 25 kg body weight (Lucas, Calder and Smith, 1959; Frape et al., 1959; Boaz and Elsley, 1962; Elsley, 1963; Nielson, 1964; Duckworth, 1965; Vanschoubrock, DeWilde and Van Spaendonck, 1965; Reid et al., 1968; Rousselow, 1973; Zimmerman and Khajarern, 1973). Similar findings have been reported by Robinson (1964) and Owen, Ridgman and Wyllie (1971) when larger pigs were restricted-fed. Topel (1971) and Young and Sharma (1973) attempted to evaluate the effect of early energy restriction per se on subsequent performance and concluded that energy intake from birth to 23 kg live weight has no effect on body composition of market pigs. Compensatory responses after a period of protein, but not energy, restriction were also demonstrated by Meade et al. (1969a,b) and Wyllie et al. (1969).

Physical and metabolic adjustments during compensatory growth

It has been shown, by most workers, that animals which were previously malnourished had greater food intake, faster rate of weight gain and utilized feed more efficiently than those of the control animals during a period of realimentation. The regulator(s) that controls these responses are not well understood. Ashworth (1969) suggested that the factors that

regulate the compensatory response must be closely related to the regulation of food intake. Mayer (1966) proposed the existence of a set of glucoreceptors located within the ventromedial nuclei of the hypothalamus. This center has subsequently been demonstrated to regulate food intake in response to blood glucose (Mayer and Thomas, 1967; Mayer and Arees, 1968). Food intake has also been demonstrated to be related to the fat content in the body (Kennedy, 1953) and skin temperature (Abrams and Hammel, 1964). Hormones may be involved in regulation of food intake. Insulin, a hypoglycemic hormone, may stimulate an increased food intake in hypophysectomized rats (Wagner and Scow, 1957). After meal consumption, the rising blood glucose and amino acids have a stimulatory effect on insulin secretion and an inhibitory effect on growth hormone secretion (Roth et al., 1963a,b; Floyd et al., 1966). Glucagon and glucocorticoids, the hyperglycemic hormones, have been demonstrated to increase blood glucose by the activations of phosphorylase (Butcher, 1968) and glucose-6-phosphatase (Weber et al., 1955) in liver. Specific dynamic action (SDA) may also affect food intake (Mickelsen, Takahashi and Craig, 1955). Previously malnourished animals have an increased SDA during realimentation (Barnes, 1968; Ashworth, 1970; Brooke and Ashworth, 1972; Barnes et al., 1973). Under similar condition, an increased basal metabolic rate (oxygen consumption) has also been demonstrated by Barnes et al. (1973). They suggested that these increased requirements for maintenance contributed approximately 50 percent of the increase of food intake during rehabilitation. No significant effect of growth hormone on feed consumption was observed by these workers.

Rate of live weight gain during rehabilitation is usually faster in the previously restricted animals than in the controls. In their reviews, Wilson and Osbourn (1960) and Allden (1970) concluded that the increased gain was partially from increases in gastrointestinal fill and partially from true increases in tissue weight. With respect to weight gains in this period, the weight increases in late maturing tissues such as muscle and fat contribute more to weight gain than those from early maturing tissues such as nerve and skeleton. Differential rates of tissue laid down during this period have been indicated by several investigators. Among these investigators, Tanner (1963), Fowler (1967), Wyllie et al. (1969) and Zimmerman and Khajarern (1973) demonstrated that lean tissue is being deposited more than fat during compensatory growth. The reverse order of differential tissue deposition was reported by Robinson (1969, 1971).

With respect to metabolic adjustments and efficiency of nutrient utilization of animals during compensatory response, the available data indicated more efficiency in nutrient utilization by previously restricted animals than by the controls. Works by Baur and Filer (1959) and Vaughan, Filer and Churella (1962) in pigs and Chan (1968) in humans suggest a faster protein metabolism in the animals fed a higher protein level when compared with those fed the lower level. Nakano and Ashida (1970) and Nakano et al. (1972) reported an increase in rat liver amino acid degrading enzymes induced by the presence of high levels of amino acids in diet. Oslage (1963) noted a linear relationship between nitrogen retention and the live weight of pigs normally fed. However, in pigs which were restricted-fed, the increase in live weight from 25 to 60 kg

was associated with a decrease in nitrogen retention. Rousselow (1973) found no differences in nitrogen retention, or in three amino acid degrading enzymes in liver, of rehabilitating pigs previously fed starter diets ranging in protein levels from 10 to 31 percent. Barnes et al. (1973) observed that rats which were previously malnourished had higher nitrogen retention than the controls when a diet containing 10 percent protein from wheat gluten was fed during the rehabilitation period. However, similar nitrogen retention was observed for these two groups when casein was used as the protein source in the rehabilitating diet. On the other hand, Lee and Chow (1965, 1968) found that rats which were previously malnourished had poorer nitrogen retention and more alpha-amino nitrogen in urine than normally fed controls.

Cell Development

General considerations

Growth of any organ may consist of an increase in number or size of cells or both (Winick and Noble, 1965; Winick, Brasel and Rosso, 1972). Study of cellular growth has been made possible by a variety of techniques, including a knowledge of the fundamental relationships of nucleic acids (DNA and RNA) to cell division and increase in cell size. The DNA content of a diploid nucleus in cells of rat brain and skeletal muscle has been shown to be constant (Enesco and Leblond, 1962; Enesco and Puddy, 1964; MacConnachie, Enesco and Leblond, 1964). The amount of DNA in rat brain and skeletal muscle has been reported to be 6.2 picograms (10^{-12} gram) per nucleus (Enesco and Leblond, 1962). A change in total DNA content of a tissue or organ, thus, indicates a change in cell number.

The weight:DNA and protein:DNA ratios are the indicators of cell size with respect to total amount of cellular material and of total amount of protein per cell, respectively (Enesco and Leblond, 1962; Winick and Noble, 1965; National Dairy Council, 1970; Winick et al., 1972). The amounts of RNA per cell and protein per cell tend to change in the same direction and, since the presence of a large amount of RNA is associated with rapid protein synthesis, the RNA:DNA ratio has been considered as an indicator of protein synthetic activity in cells (Winick and Noble, 1965).

Studies in rats have indicated that in many organs and tissues there are three phases of cell development during growth: (a) hyperplasia, when cell numbers are increasing and cell size remains constant; (b) hyperplasia and concomitant hypertrophy, when there is a decrease of cell division and a beginning of increase in cell size; and (c) hypertrophy, when all cell growth occurs by enlargement of individual cells (Enesco and Leblond, 1962; Winick and Noble, 1965; Winick, 1968).

Enesco and Leblond (1962) studied growth in the whole body, as well as tissues and organs, of the Sherman rat from birth to 90 days of age. For the first 17 days after birth, rapid growth was by addition of new cells in epididymal fat and of new nuclei in skeletal muscle. The cell size, as indicated by weight:DNA ratio, remained constant in this period. In the period between 17 to 48 days after birth, addition of DNA continued in all tissues but at a reduced rate, while cell size increased rapidly. After 48 days of age, the addition of new cells was decreased drastically in epididymal fat and skeletal muscle and stopped completely in skin and thymus.

In their comprehensive study on cell growth in the Sprague-Dawley rat, Winick and Noble (1965) found a progressive increase in DNA (cell number) both for the whole animal and for individual organs from 10 days after conception to at least 13 days postnatally. The size of cells was constant during the period. As the postnatal growth proceeded, tissue DNA synthesis decreased, and the rate of decrease depended on the tissue in question. For example, DNA synthesis in brain had stopped by the 20th day after birth, whereas the synthesis in skeletal muscle continued throughout the experimental period of 44 days. Total organ RNA increased during growth; however, the RNA:DNA ratio remained essentially constant. Tissues actively engaged in protein synthesis, such as skeletal muscle, heart and liver, were rich in RNA, whereas other tissues were low in RNA. Protein:DNA ratio increased progressively in all tissues during the period of 4 to 44 days postnatally. They suggested that during early growth RNA reached its final amount per cell even in face of rapid cell division and that the amount of RNA was sufficient to sustain normal rate of protein synthesis.

Muscle growth

Muscle fibers form by the fusion of multinucleated cells. The nuclei within the muscle cells are thought not to divide mitotically or amitotically (Stockdale and Holtzer, 1961). The nuclei proliferation in the multinucleated cells is achieved by the fusion of mitotic-mononucleated myoblasts with the existing myotubes (Carpers, 1960; Stockdale and Holtzer, 1961). Satellite cells, the mononucleated cells that are wedged between the muscle plasma membrane and the myotubes (Mauro, 1961; Shafiq,

Gorycki and Mauro, 1968), are thought to be resting myoblasts available for the supply of new nuclei (Bischoff and Holtzer, 1969).

Growth of muscle, similar to all other tissues, is accompanied by increments in the size and number of cells. Since the size of all cells is limited, the increments of cell number usually affect growth more than does the increase in cell size (Montgomery, 1962; Enesco and Puddy, 1964; Cheek, 1968; Moss, 1968a,b; Cheek and Hill, 1970; Cheek et al., 1971).

The cytoplasmic volume of muscle cell per nucleus is limited. In studies of Moss (1968a,b), it was found that the mean cross-sectional area of fibers and the total number of nuclei in pectoral and gastrocnemius muscles of chickens maintained a constant ratio during growth between 0 and 266 days of age. He suggested that there was a maximum cytoplasmic volume that could be controlled by a given nucleus.

The number of muscle fibers was believed to be fixed at birth in man (Goss, 1966), sheep (Joubert, 1956), pig (McMeekan, 1940; Staun, 1963) and chicken (Smith, 1963). It was believed that postnatal growth of skeletal muscle almost entirely resulted from hypertrophy of existing fibers, accompanied by an increase in the amount of extracellular material.

It is currently believed that multinucleation plays an important role in postnatal growth of skeletal muscle. Enesco and Puddy (1964) demonstrated, from their histometric and chemical studies of three muscles in rats from 15 to 94 days of age, that the number of individual muscle fibers did not increase but the number of nuclei, as indicated by DNA content, increased 2-fold in biceps and increased 3- to 4-fold in gastrocnemius muscles. A similar finding was reported for quadriceps

muscle (Gordon, Kowalski and Fritts, 1966). According to Enesco and Puddy (1964) and Gordon et al. (1966), the hyperplasia of rat muscle cell nuclei continues until at least 90 days after birth. In contrast, Cheek et al. (1968), who calculated the total muscle nuclei populations from the DNA content of a single muscle sample, stated that hyperplasia of muscle cell nuclei in normal male Sprague-Dawley rats was terminated at about 56 days of age.

Robinson (1969) reported that the postnatal active hyperplasia in pig skeletal muscle, as indicated by an increase in total DNA contents of the triceps and semitendinosus, occurred to at least 100 days of age. Evidence of postnatal hyperplasia of muscle nuclei was also reported in human by Cheek (1968). He calculated total muscle nuclei population, by making use of DNA content in a single muscle sample and 24-hour creatinine excretion, and found that the total muscle nuclei number in normal children represented a 14-fold increase from 2 months to 16 years of age.

Adipose tissue growth

The basic difficulty in studying cellularity of adipose cells is the accurate measurement of the number and the lipid content of adipose cells (Goldrick, 1967). The difficulty may be caused by the capability of adipose tissue in expanding and contracting markedly under different conditions of age, endocrine status and energy balance (Benjamin et al., 1961; Hausberger, 1967; DiGirolamo and Rudman, 1968). Various methods, including the use of DNA content (Peckham, Entenman and Carroll, 1962; Zingg, Angel and Steinberg, 1962), microscopic measurements of isolated

stained cells (Gliemann, 1967; Bray, 1969; DiGirolamo, Mendlinger and Fertig, 1969) and the electronic counter (Hirsch and Gallian, 1968), were developed. Rodbell (1964) pointed out that approximately 35 percent of DNA yielded in adipose tissue extraction was contributed by nonadipose cells. Hence the DNA analysis on adipose tissue may not provide meaningful measures of adipose cell number.

Adipose tissue of an animal grows by an increase in both number and size of its fat cells (Hirsch, Knittle and Salans, 1966). The adipose cell number reaches its peak by 10 to 15 weeks of age in rats (Hirsch and Han, 1969) and may not be before 25 years of age in human (Bray and Gallagher, 1970). After the adult value of adipose cell number is reached, it remains constant and subsequent growth is accomplished by continued deposition of lipids in existing cells (Hirsch et al., 1966). DiGirolamo and Mendlinger (1971) demonstrated a species difference in rate and pattern of the increases in cell number and cell size in rat, mouse and guinea pigs from 5 weeks to 1 year of age. Increased adipose cell size with age has also been observed in calves (Tinyakov et al., 1968).

In humans, Hirsch et al. (1966) indicated that there are two types of obesity. The first is characterized by marked increase in adipose cell number with a lesser increase in cell size. This type of obesity is demonstrated to occur in early life (Knittle and Hirsch, 1968). The second type of obesity is associated with increase in adipose cell size but normal number of fat cells (Salans, Horton and Sims, 1970). Disorders of carbohydrate, insulin and triglyceride metabolisms are reported in both forms of obesity (Salans and Wise, 1970). Baker (1969) studied

adipose tissue obtained at necropsy of 37 normal well-nourished humans ranging in age from infancy to 71 years. His results indicated that at about 4 months of age, adipose tissue constitutes as much as 50 percent of total body weight, but lipid content is lower and the number of cells per gram of wet adipose tissue is higher than later in life.

Nutrition and Cell Development

Prenatal nutrition and subsequent cell development

Zeman and Stanbrough (1969) fed rats semipurified diets containing either 30 or 6 percent casein during pregnancy. DNA, RNA and protein in the whole carcass of 16-day-old fetuses, and in liver, heart, kidney, brain and the remaining carcass of 18- and 20-day-old fetuses, and in newborn young were determined. They showed that the primary effect of maternal deficiency on fetal growth was a decrease in cell number during the last four days of gestation while normal cell size was retained.

Results similar to those of Zeman and Stanbrough (1969) were reported by Zamenhof, Van Marthens and Grauel (1971) who fed albino rats a protein-free diet during five periods of pregnancy: days 0 to 10, 10 to 15, 13 to 18, 15 to 20 or 10 to 20, and a normal diet during the remaining time. They observed significant decreases in cerebral cell number and protein content of neonates in all groups when compared to a control group. They suggested that fetal amino acid deficiency was unlikely to occur before day 15 of gestation. The reduced protein increment of fetuses in mothers fed protein-free diet before day 15 was attributed to hormonal control and placental deficiency. Estrogen and progesterone might play important roles in this case.

In pigs, Pond et al. (1969a,b) studied the effects of dietary protein deprivation during various intervals of gestation in the gilt on the subsequent growth and on nucleic acid content of brain and muscle of the progeny during young adulthood. They found that dietary protein deprivation of the gilt throughout pregnancy resulted in reduced birth weight and postnatal weight gain of the progeny but did not permanently affect DNA content of cerebrum or cerebellum. RNA concentration and total RNA in these two organs may be decreased, suggesting depressed protein synthetic activity. DNA concentration of Longissimus dorsi muscle at 90 kg body weight was not affected by treatment, but RNA:DNA was significantly reduced in progeny of protein-deprived gilts. Robinson (1969) fed sows a balanced diet throughout pregnancy and lactation (C), or 50 percent restriction during pregnancy (RP), or 50 percent restriction throughout pregnancy and lactation (PL). Pigs were killed at predetermined ages ranging from birth to 150 days and DNA, RNA and total protein were determined in the triceps and semitendinosus. He found that, as pigs grew older, the DNA and RNA concentrations in both muscles decreased rapidly to stable levels at about 80 days of age. Total amount of DNA (cell number), however, increased to a stable level at 30, 60 and beyond 100 days for pigs from PL, RP and C groups, respectively. He suggested that hyperplasia in pigs from sows being restricted fed during pregnancy and lactation terminated much earlier than that in pigs from sows normally fed throughout or during lactation alone. He also suggested that hyperplasia in triceps and semitendinosus muscles of pigs from normally fed sows could occur to at least 100 days after birth.

Postnatal nutrition and subsequent cell development

The most common methods employed in altering the nutritional status of neonatal rats are: varying the number of pups nursing a single mother, restricting the protein intake in the lactating mother to reduce the quantity of milk produced without altering its composition, and varying the time allowed the animals to nurse per day (Winick et al., 1972). Pre- and postnatal nutrition are equally important to subsequent development in rat brain. Winick, Fish and Rosso (1968) and Winick (1970a,b) demonstrated that pups subjected to either prenatal or postnatal malnutrition showed a 15 to 20 percent reduction in total brain cell number at birth or at weaning, respectively. The pups which were malnourished during both periods showed a 60 percent reduction in cell number by weaning.

The reduced cell population present at birth of neonates from gestating-protein-deficient mothers is not corrected by the increased food intake during the suckling period. Zeman (1970) allowed pups of dams which were fed diets containing either 30 or 6 percent casein during gestation to suckle from nondeficient mothers in litters of eight until weaning or in litters of four at 7 days of age to weaning. She observed that at 7, 14 and 21 days of age, young of protein-deficient dams, suckled in litters of eight, had significantly decreased organ weights, DNA, RNA and total protein which persisted from birth but did not become more severe. When litters were cut to four in order to increase food intake the livers, kidneys and hearts increased in weight and cells increased in size, but there were no significant changes in the number of cells in these organs. In the thymus, however, the number of cells increased and cells decreased in size, while in the brain, the weight,

DNA content and apparent cell size were unaffected. Results somewhat contradictory to that of Zeman (1970) were reported by Winick (1971), who observed that the newborn pups of protein-restricted mothers were able to overcome the deficit in total brain cells if they were raised in litters of three on normal foster mothers until weaning. He suggested that the postnatal increase in cell number might occur in areas different from those most affected in utero.

The effect of postnatal nutrition, separately from the prenatal nutrition, on subsequent cell development in various organs was extensively investigated in Winick's laboratory. Winick and Noble (1966) imposed a 50 percent feed restriction for 21 days to rats at birth, at 21 days or at 65 days of age and then refed normally until they were 133 days old. Total weight, protein, RNA and DNA were measured in various organs including the gastrocnemius at the end of each restricted period, as well as at the 133rd day. When compared to control animals, they found that malnutrition from birth to day 21 resulted in a permanent reduction in cell number (DNA), without alteration in cell size (weight:DNA or protein:DNA), of all organs studied. Also, malnutrition from days 21 to 42 resulted in permanent reduction in cell number of all organs except brain and lung. In brain and lung only cell size, not cell number, was reduced and was restored when the rats were refed normally. Finally, malnutrition from 65 to 86 days of age resulted in decreased cell size with retention of cell number in all organs except spleen and thymus. After the refeeding, all organs in those animals recovered normal cell size. In subsequent experiments, Winick and Noble (1967) allowed pups to be nursed in litters of three to six per mother to wean at 21 days of age. At weaning, they

observed that the organs of these animals contained more cells than those of control rats. Winick et al. (1968) raised pups in litters of eighteen from birth to 9 days of age, and from 9 days to weaning litter size was cut to three. They reported that the cell number in various organs of these rats, that was reduced by undernutrition during the first 9 days of life, was restored by the increased food intake in the second period. From these data, they concluded that cellular effects of nutritional status depend on phase of growth of the animal at the time the restriction occurs. The variation of nutritional status in neonatal period affected the rate of cell division and final number of cells in all organs. Unless refeeding was initiated early in hyperplastic growth period, the normal number of cells would not be attained. Nutritional variation imposed somewhat later caused permanent alteration only in the later developing tissues, whereas in the early developing tissues, in which hyperplastic growth had already ceased, the effects were reversible.

With respect to muscle cell growth in rats, the restriction of protein or of energy, or of protein and energy, acts differently. The total food intake may play an important role here since the voluntary caloric intake of rats decreased when presented with a low-protein diet (Cheek et al., 1971). As indicated by Winick and Noble (1966), restriction to 50 percent of protein plus caloric intakes in postweaning rats resulted in permanent growth retardation of the gastrocnemius with a reduction of total DNA, RNA and protein contents. The total cell number, RNA, protein and weight of the muscle were not corrected by refeeding, while the muscle cell size was subsequently normal.

Robinson and Lambourne (1970) fed mice to grow normally to predetermined weights at which time a control group was killed. Others were treated by means of feeding, to grow, to maintain or to deplete body weight for 2 weeks and were then killed. Further groups were permitted to grow normally for 2 weeks following maintenance or depletion. With this design, they were able to obtain mice of different empty body weights and growth rates. They found that, while the total DNA content of hind limb was essentially constant, the nitrogen:DNA ratio was a linear function of the empty body weight and of the total nitrogen content of the body. The RNA concentration in the muscle, however, was inversely related to the empty body weight. During refeeding, the RNA concentration was a linear function of growth rate.

In an attempt to separate the effects of protein restriction from energy restriction in weaning rats, Hill et al. (1970) assigned 23-day-old rats to one of four dietary treatments to reach 48 days of age. The dietary treatments were combinations of high (27 percent) or low (6 percent) casein and normal or restricted (66 percent) energy. Rats in the high-protein, restricted-energy group attained approximately 60 percent of the control values for body weight, quadriceps mass and total muscle nuclei with normal muscle cell size (protein:DNA). The rats in both low-protein groups, however, were found to consume identical amounts of energy per rat per day. The caloric intake in these groups was adequate for body size but inadequate for age. The cellular response thus reflected the restrictions in protein plus calories. They found that the number of muscle nuclei in these groups was approximately 33 percent of that in control animals. The muscle mass, muscle cell size (protein:DNA) and

RNA:DNA ratios were significantly lower than the controls. They consequently concluded that caloric restriction *per se* retarded muscle cell multiplication without affecting cell size, while a protein and calorie deficiency retarded both DNA replication (cell number) and protein synthesis (cell size).

The effect of caloric restriction *per se* on cellular response of postweaning rats has been reported by several investigators. Elliot and Cheek (1966) compared cellular growth of postweaning rats being fed in a hypoxia chamber containing 12 percent oxygen or pair-fed outside the chamber. The protein consumption of hypoxic rats and their pair-fed mates was approximately enough for the requirement while their energy consumptions were 75 percent of the controls. After a 3-week treatment period, they found a reduced DNA accumulation with normal protein:DNA ratio in the thigh muscle of the restricted groups when compared to the controls. Similar findings were demonstrated in their subsequent experiments (Elliot and Cheek, 1968; Graystone and Cheek, 1969). In addition, Elliot and Cheek (1968) observed significant, but incomplete, compensatory growth in the calorie restricted rats when they were fed *ad libitum* in room atmosphere during the 6th week of the experiment.

Caloric restriction during postweaning period has been demonstrated to retard the age at which maturation in terms of hyperplasia was reached. Durand, Fauconneau and Penot (1967) found, from their 15-week caloric restriction study in rats, that the increase in nuclei number was retarded without affecting muscle cell size (protein:DNA) during early weeks of restriction. As time progressed the muscle DNA content of the caloric restricted rat continued to increase even when that in controls had stopped.

Postweaning protein deficiency has been shown to affect cellular development in rats by Mendes and Waterlow (1958). They compared DNA, protein and mass of the gastrocnemius in weanling rats fed a low-protein, high-carbohydrate diet for 28 days and then an 18 percent protein control diet for 10 and 20 days to rats fed a control diet throughout the experiment. They observed that protein deficiency caused complete retardation in muscle growth, DNA accumulation and muscle protein content per unit of DNA by the 28th day. Upon refeeding, muscle protein synthesis and DNA accumulation reached their maximum rates after a short lag period. More recently, Dickerson, Hughes and McAnulty (1972) fed 24-day-old rats isocaloric diets containing either 5 or 25 percent casein for 28 days. At the end of the treatment period, the animals that were not slaughtered were fed the 25 percent casein diet to 140 days of age. They found that the low-casein diet caused a complete cessation of growth of the quadriceps. The muscle DNA concentration and total muscle DNA of the deficient rats were lower than those of the controls. Upon rehabilitation, the DNA concentration and total DNA in the muscle of the former were similar to those of the latter; however, the muscle weight was significantly lower than that of the controls.

Strunz and Lenkeit (1964) fed 2-week-old pigs diets with high or low protein contents to 8 weeks of age. From each treatment, at 1-week intervals, a pig was killed for nucleic acid and protein determinations in various organs including skeletal muscle. They found that the DNA: nitrogen ratios in skeletal muscle, liver and kidney were higher in pigs fed low-protein diet than in pigs of the same age on high-protein diet. The ratios in all tissues except liver fell with age. In both groups,

RNA values were closely correlated with nitrogen content of tissues, but the RNA:nitrogen, as well as RNA:DNA ratio, were higher in pigs given more protein. Subsequently, Strunz, Meyer and Fricke (1966) conducted a similar experiment in which more pigs were involved. Diets containing 37 or 11 percent crude protein were fed from 1 week of age. They found that total liver cell number (DNA) rose linearly with liver nitrogen content and was, therefore, lower in low-protein pigs. The cell size (nitrogen: DNA), however, remained constant. In kidney, heart and skeletal muscle the nitrogen:DNA ratio, as well as the plasma:nucleus ratio, rose linearly as the size of organ increased. Again, the nitrogen:DNA ratio was higher in high-protein pigs than the others. From these data, they concluded that, in pigs, liver grew by hyperplasia alone while the other tissues studied grew by a combination of hypertrophy and hyperplasia. More recently, Moser, Peo and Cunningham (1972) showed a linear decrease of DNA concentration in gracilis samples as protein level in diets, fed to early weaned pig for 35 days, increased from 12 to 20 percent. However, RNA:DNA ratios increased as the protein levels in diets increased.

Nutrition and adipose tissue development

Nutritional status in early life has been demonstrated to affect the subsequent development of adipose tissue in rats. Overnutrition during the suckling period is associated with a higher number of adipose cells and more lipid content per cell, when compared with rats which were restricted fed. Knittle and Hirsch (1968) demonstrated, in rats that were suckled in litters of four or twenty-two and subsequently fed ad libitum, that the rats that were fed a high plane of nutrition had

more adipose cells at 5 weeks and higher lipid per cell at 10 weeks after weaning, while the rates of glucose incorporation into CO_2 and triglycerides on a per cell basis were similar. They concluded that nutrition during early life can permanently affect the cell number and total metabolism in rat adipose tissue. Subsequently, Knittle (1972) showed that caloric restriction of nursing mothers produced a reduction of adipose cell size without affecting cell number and the small cell size disappeared when the pups were subsequently fed ad libitum. On the other hand, maternal protein restriction reduced the epididymal pad size permanently by the reduction of cell number.

In humans, the effect of obesity on cell size and number follows that in rats. The obese person has nearly three times the normal number of fat cells and 0.9 mcg lipid per cell compared with 0.6 mcg per cell of control (Rabinowitz, 1970). Cheek et al. (1970) speculated that in the obese male, the superimposition of high levels of circulating insulin on androgen enhances the growth of collagen and of adipocytes in adipose tissue mass. In the obese female, estrogens, which retard cell number increase, are possibly suppressed by androgens and thus allow the latter and growth hormone to exert maximal effects on tissue growth.

Lee, Kauffman and Grummer (1972a,b) demonstrated that pigs which were severely restricted-fed during a 4-week suckling period and subsequently fed normally to 24 weeks of age or to 80 kg body weight had fewer and smaller adipose cells in the interfascicular spaces when compared with those of controls. However, the restriction of feed intake during early life did not affect the adipose cell number or size of subcutaneous, visceral and bone fat.

EXPERIMENTAL PROCEDURE

The research reported herein is on file in the Swine Nutrition Section of the Animal Science Department at Iowa State University, Ames, Iowa, as Experiments 7107, 7119 and 7215. The experiments were conducted at the Iowa State University Swine Nutrition Farm. The pigs were crossbreds of Hampshire, Landrace, Poland China and Yorkshire ancestry. All animals were weighed, eye teeth were clipped and ears were notched during the first 24 hours after birth. Male pigs were castrated during their first week of life. The pigs were weaned between 21 and 26 days of age.

At the beginning of the experiments, all pigs were treated with Furacin[1] for prevention of diarrhea. Subsequently, they were individually treated as needed with Furacin and Bactrovet[2]. Throughout the experiments all animals were housed individually and water was supplied continuously.

Data Analysis

All data were statistically analyzed and F tests were conducted to determine differences among treatments. Missing values were calculated, according to methods described by Snedecor and Cochran (1967) for pigs that died before the end of experiments.

The word "protein" to be mentioned in results and discussions implies the protein that was determined by method of Lowry *et al.* (1951) when nucleic acids were discussed; otherwise, it means crude protein (N X 6.25) which was determined by Kjeldahl method (A.O.A.C., 1965).

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[1]Eaton Laboratories, Norwich, New York.

[2]Pitman-Moore, Inc., Fort Washington, Pennsylvania.

Chemical Analyses

Adipose tissue

Adipose cell number The method used for the determination of adipose cell number was developed and referred to as Method II by Hirsch and Gallian (1968). Tissue shreds, each 5 to 10 mg (total less than 500 mg), were washed free from adherent fat in warm isotonic saline. They were then transferred into 30 ml plastic vials containing Kreb-Ringer bicarbonate buffer solution (pH 7.4) with added bovine serum albumin (4 percent), 3 mM glucose and collagenase enzyme from Clostridium hystolyticum[1] at a concentration of 10 mg/g of adipose tissue. The vial and its contents were incubated for 1 hour at 37° C. At the end of the hour, the vial was shaken on an Eberbach shaker at a rate of 160 cycles/minute for 1 to 3 minutes maintaining the samples at 37°C. The vial and its contents were cooled for 1 hour in a cold room at 4°C. About 10 ml of cold fixative (0.25 percent trichloroacetic acid and 6.25 percent glutaraldehyde in water) was added and allowed to react for an hour. The subsequent procedure was performed in a cold room at 4°C. The fixed, free cells were separated from tissue and debris by passage through a "Nitex" nylon screen[2] having a pore size of 250 microns. The filtrate was then passed through another screen having a pore size of 25 microns. The trapped cells were quickly washed from the second screen into a plastic beaker with 100 ml of cold, isotonic saline. A 25 ml aliquot of the TCA-glutaraldehyde fixed cell suspension was withdrawn into a siliconized

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[1] Worthington Biochemical Corporation, Freehold, New Jersey.

[2] Tobler, Ernst and Traber, Inc., 71 Murry Street, New York, New York.

glass pipette and stored in a plastic vial for carboxyl ester bond determination. The remaining suspension was then processed for cell counting. A Coulter electronic counter[1] with a 400 microns aperture was used. Under gentle agitation to maintain an even suspension, five separate counts per 2 ml were determined for each suspension.

Carboxyl ester bonds The method used for preparation of samples for determination of fatty ester in TCA-glutaraldehyde-fixed cells was described by Hirsch and Gallian (1968). A 25 ml aliquot of TCA-glutaraldehyde-fixed cell suspension was emptied into a fritted glass filter tube (pore size 0.9 to 1.4 microns) containing a loose filter bed of celite particle (previously washed in chloroform and methanol). The plastic vial was repeatedly washed with cold saline and the washings were delivered to the filter bed. The bulk of the filter content was emptied into a glass-stoppered tube and the remainder was rinsed into the tube with methanol. Sufficient amount of chloroform was added to make the final composition of the mixture of chloroform and methanol 2:1. After the addition of 0.2 volume of distilled water and separation of the phases, an aliquot was removed from the chloroform layer for lipid determination by measurement of carboxyl ester bonds (Rosenthal, Pfluke and Callerami, 1959).

Total lipid content in adipose tissue also was determined by measurement of carboxyl ester bonds (Rosenthal et al., 1959) in the chloroform-methanol (2:1) extraction as described by DiGirolamo et al. (1971).

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[1]Model F. Coulter Electronics, Hialeah, Florida.

Chemical composition of the body

Samples for determination of chemical composition were prepared as described previously (Khajarern, 1971). The moisture, ether extract, ash and total nitrogen determinations were made according to methods described in A.O.A.C. (1965). Each component was expressed as a percentage of fresh tissue.

Muscle fiber diameter

The estimation of muscle fiber diameter was based on a technique developed by Hegarty and Naude (1970). A transverse muscle "chip" of about 25 mg was cut, while frozen, and placed in a 1 ml stainless steel homogenizing chamber containing 0.5 to 0.8 ml of isotonic saline solution. The fibers were teased apart by homogenization for 5 seconds with a Virtis 45 homogenizer at the slowest speed.

A 0.3 ml aliquot of the slurry was placed in the deep well of a culture slide[1] for immediate observation under microscope. Measurement of the width of muscle fibers was made by means of an ocular micrometer inserting into the eye piece (10X). A 10X objective lens was used. By means of a stage micrometer[2], the calibration of the ocular micrometer was determined to be 6.51 units per millimeter. The same micrometer and microscope were used for all measurements and illumination was provided artificially.

The mean fiber diameter was calculated from the width at the middle of 100 fibers that were observed while moving the slide across from left

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[1]Matheson Scientifics 50680-10, 2.54 cm cylinderical concavity.

[2]A. H. Thomas Corporation, No. 6852-A.

to right and then, at a slightly lower level, back again to the left. This was done until the required 100 fibers were measured. A second slide was occasionally prepared to get enough measurements.

Nucleic acids and protein

A modified Schmidt-Thannhauser (1945) method, as recommended by Munro and Fleck (1966), was used for the determination of the RNA and DNA in muscle. Triplicate 1 g muscle samples were homogenized in 5 volumes of ice-cold distilled water at 0°C for approximately 3 minutes. A 4 ml aliquot of the homogenate was transferred into a 15 ml centrifuge tube. DNA, RNA and protein were precipitated by adding 2.5 ml of ice-cold 0.6 N $HClO_4$ to each tube. The resulting suspension was mixed, allowed to stand in an ice bath for 10 minutes and centrifuged at 8000 g for 10 minutes. The supernatant was discarded. The precipitate was washed twice with 5 ml of cold 0.2 N $HClO_4$ and the excess acid was drained off by inverting the tube briefly over a filter paper. The precipitate was resuspended in 2 ml ice-cold distilled water, followed by 2 ml of 0.6 N KOH. The suspension was incubated in a water bath at 37°C for 60 minutes. The suspension was cooled in ice and 2.5 ml of ice-cold 1.2 N $HClO_4$ was added to reprecipitate DNA and protein. After standing for 10 minutes in the ice bath, the suspension was centrifuged at 9000 g for 10 minutes and the supernatant containing the RNA fraction was transferred into a 50 ml volumetric flask. The precipitate was washed twice with 5 ml of ice-cold 0.2 N $HClO_4$ and the washings were added to the RNA fraction. After adding another 5 ml of ice-cold 0.2 N $HClO_4$, the RNA fraction was made to a final volume of 50 ml with distilled water. The resulting RNA fraction was in 0.1 N $HClO_4$.

RNA standard solution was prepared by hydrolyzing 25 mg of bovine pancreas RNA[1] in a solution made up of 2.5 ml of distilled water and 2.5 ml of 0.6 N KOH at 37°C for 60 minutes. The hydrolyzed RNA was then made to a final volume of 50 ml with 0.1 N $HClO_4$. A blank solution made up of 2.5 ml distilled water and 2.5 ml 0.6 N KOH was similarly prepared. By appropriate dilutions RNA concentrations of 5, 10, 15, 20 and 25 µg/ml were prepared from the hydrolyzed RNA solution.

RNA concentrations in the samples were then determined by ultraviolet absorption at 260 nm and read against a standard curve obtained from the RNA standard solutions. Protein in RNA was determined by the phenol-biuret method of Lowry et al. (1951). The RNA was corrected for protein by subtracting 0.001 OD per mcg protein.

The DNA-protein precipitate obtained by acidifying the alkaline digest was suspended in 2.5 ml of distilled water followed by 2.5 ml of 0.6 N KOH and dissolved by warming at 48°C. After adding 3.5 ml of 0.3 N KOH, the solution was brought to final volume of 25 ml with distilled water, resulting in a 0.1 N KOH solution. A 2 ml aliquot of the solution was used for DNA determination by the indole reaction as described by Ceriotti (1952).

DNA standard solutions were prepared by dissolving 25 mg of calf thymus DNA[1] in 50 ml of distilled water with the aid of 0.1 N KOH. Appropriate dilution with 0.1 N KOH was made to obtain DNA concentrations of 5, 10, 15, 20 and 25 µg/milliliter. DNA concentrations in the samples were determined by spectrophotometry at 490 nanometers.

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[1]Sigma Chemical Company, P.O. Box 14508, St. Louis, Missouri.

Total protein content in the homogenates was determined by the phenol-biuret method of Lowry et al. (1951).

Duplicated homogenates of the whole body, ground in 5 volumes of ice-cold distilled water, were used for determination of total nucleic acid and protein content in Experiment 7215.

Plasma analyses

Ten to 15 ml of blood was collected in heparinized (1000 IU/ml) isotonic saline solution. After centrifugation, plasma samples were frozen and stored at -10°C until analyzed. A Technicon Auto-Analyzer was used for determinations of glucose (Hoffman, 1937), urea nitrogen (Marsh, Fingerhut and Miller, 1965) and alpha-amino nitrogen (Palmer and Peters, 1966).

Experiment 7107

Effect of Energy Level in Starter Nutrition on Nucleic Acid and Protein Content in the Lean Tissue of Market Hogs

Objectives

In previous experiments (Khajarern, 1971) pigs whose energy intake was restricted during the starter period (4.6 to 22.7 kg body weight) contained more body protein at market weight than pigs that were full-fed. Cheek and Hill (1970) developed a hypothesis that deficiency of energy in rats primarily interrupts cell growth by reducing net DNA production while deficiency of protein primarily interrupts cell growth by reducing net protein synthesis. The purpose of this study was to determine whether an energy restriction during the starter period had an effect on the

cellularity (nucleic acids, protein content and their relationships) of lean tissue of pigs at market weight.

Procedure

Twenty-seven 19- to 26-day-old pigs averaging 5.5 kg body weight were used. Littermates were allotted at random to one of three dietary treatments. A randomized block design of nine replications was used. The dietary treatments during the 45-day starter period were: 12 percent crude protein, corn-soybean meal diet fed at 100 percent of a previously established feeding scale (Khajarern, 1971); 14.4 percent crude protein diet fed at 83.3 percent full-feed intake; and 18 percent crude protein diet fed at 66.7 percent full-feed intake. With this feeding scale, all pigs received 4.32 kg of protein and pigs of Treatments 1, 2 and 3 received 107, 89 or 71 Mcal of metabolizable energy, respectively, in 45 days. Starter diets were isocaloric and equal in protein quality. Pigs were penned and fed individually. Composition and calculated analyses of the experimental diets are given in Tables 20, 21, 26 and 27.

After the starter period, all pigs were fed a 16 percent protein grower diet to 57 kg body weight and a 12 percent protein finisher diet until pigs were slaughtered at about 90 kg body weight. All pigs were slaughtered at the Iowa State University Meat Laboratory. Half-carcasses were frozen and stored at -14°C until sampled for carcass analysis. A small L. dorsi sample was taken from the area of the 8th to 10th rib of each carcass. The content of DNA, RNA and protein in the L. dorsi was determined.

Results

Summaries of nucleic acid and protein contents in the L. dorsi are given in Table 1. There were no treatment differences in the concentrations of DNA, RNA and protein or RNA:DNA, or protein:DNA ratios of the L. dorsi samples at market weight. Summaries of total nucleic acid contents in the body are presented in Table 2. The values were calculated by the use of protein:DNA ratio and total protein in the empty body, assuming the protein:DNA ratio of L. dorsi to be representative of the body. There were no significant differences among treatments in DNA or RNA content in the empty body of market pigs.

Table 1. Experiment 7107 - Effect of starter energy intake on nucleic acid and protein contents in Longissimus dorsi

	Treatments			C.V.
Energy, Mcal	107	89	71	%
DNA, mg/g	0.281	0.283	0.281	4.80
RNA, mg/g	0.720	0.754	0.722	9.98
Protein, mg/g	171.97	180.08	168.73	9.99
RNA:DNA	2.58	2.67	2.58	8.69
Protein:DNA	613.4	637.6	602.0	7.47

Table 2. Experiment 7107 - Effect of starter energy intake on total empty body nucleic acid content[a]

	Treatments			C.V.
Energy, Mcal	107	89	71	%
DNA, g	17.78	18.34	19.38	8.89
RNA, g	48.29	48.74	49.76	9.78

[a]Calculated from empty body protein (N X 6.25) and protein:DNA ratio of L. dorsi.

Experiment 7119

Effect of Starter Nutrition on Compensatory Performance and Chemical Composition of Body and Lean Tissue of Market Hogs

Objectives

Compensatory response of weight gain, feed efficiency and chemical composition of swine occurs after a period of energy and protein restrictions in the starter period. However, no fully acceptable explanation of how and when the compensatory performance occurs has been given. The purposes of this experiment were: 1) to determine whether restriction of starter energy and protein intakes has compensatory effects on rate of weight gain, efficiency of feed and protein utilization and fasting plasma metabolite patterns during the starter, early grower and early finisher periods; 2) to determine the effect of the restriction of starter nutrition on body composition and cellularity of lean tissue at market weight; 3) to determine whether compensatory growth could be understood in view of cellularity of lean tissue.

Procedure

Twenty pigs, averaging 5.4 kg body weight, were used in this experiment. A 2 X 2 factorial arrangement in a randomized complete block design with five replications was employed. Four littermates were allotted at random to one of four dietary treatments. The dietary treatments applied from 5.4 kg body weight to the end of a 44-day starter period were: an 18 percent crude protein, corn-soybean meal diet fed at 100 percent of full-feed intake; a 27 percent crude protein diet fed at 66.7 percent of full-feed intake; a 12 percent crude protein diet fed at 100 percent of

full-feed intake; and an 18 percent crude protein diet fed at 66.7 percent of full-feed intake. With this feeding regime, the pigs in high-protein treatments received 6.79 kg protein and those in low-protein treatments received 4.53 kg protein during the starter period. The pigs in the full-fed treatments received 114.7 Mcal of metabolizable energy and those in the restricted-fed treatments received 74.5 Mcal of metabolizable energy. Pigs were individually fed. Diets were isocaloric and equal in protein quality. After 44 days of starter period, all pigs were self-fed a 16 percent crude protein, corn-soybean meal grower diet until the average weight of pigs in each replication reached 57 kilograms. From 57 to 93 kg body weight the pigs were fed a 12 percent crude protein, corn-soybean meal finisher diet. Composition and calculated analyses of the diets are given in Tables 22, 23, 26 and 27.

Nitrogen balance studies were conducted during the last 8 days of starter and the first 8 days of grower and finisher periods. Five-day collection was made after a 3-day adjustment in metabolic crates for each period. During the adjustment and collection periods, pigs were fed according to the feeding scale in the starter period and at 4.5 percent of body weight in the grower and finisher periods. The daily feed was divided into two equal feedings at 8 a.m. and 4 p.m. Urine was collected in bottles containing 25 ml concentrated HCl and 25 ml toluene. At the end of collection periods, total urine volume was measured and a 5 percent subsample was taken for nitrogen determination. Feces were collected daily and stored frozen in plastic bags. At the end of the collection periods, total feces were weighed and a sample freeze-dried. Refused feed was collected, forced-air-oven dried and weighed. Nitrogen

was determined on feed, dried feces, urine and the refused feed samples.

Blood samples were taken on the second day after the pigs were removed from metabolic crates. The bleedings were made at 0, 2, 4, 8 and 16 hours of a fast. The plasma samples were analyzed for glucose, urea nitrogen and alpha-amino nitrogen.

As each pig reached approximately 93 kg body weight, it was slaughtered at the Iowa State University Meat Laboratory. Samples for determination of chemical body composition and nucleic acids were collected as described previously.

Results

Summaries of average daily weight gain and feed:gain ratio are given in Table 3. During the 44-day starter period, pigs fed the higher protein intake grew faster ($P<.01$) and utilized feed more ($P<.01$) efficiently than those fed less protein. Pigs that were restricted-fed grew slower ($P<.01$) and had lower ($P<.01$) feed:gain ratios than pigs that were full-fed. There were significant ($P<.05$) protein x feeding level interactions with respect to average daily gain and feed:gain ratio in this period. Restriction of feed intake at the higher protein intake depressed average daily gain and improved feed:gain more than when the restriction was applied at the lower protein intake. From the end of the starter period to 57 kg body weight (grower period), pigs that were previously restricted-fed gained weight faster ($P<.05$) and utilized feed more ($P<.05$) efficiently than the full-fed groups. No significant effects of protein intake or protein x feeding level interaction were

Table 3. Experiment 7119 - Effect of starter nutrition on average daily gain and feed:gain ratio

	Treatments				
	1	2	3	4	
Protein, %	18	27	12	18	C.V.
Level of feeding, %	100	66.7	100	66.7	%
Starter					
ADG, kg	0.44	0.32	0.39	0.31	3.77[a,b,c]
Feed:gain	1.85	1.69	2.09	1.79	3.38[a,b,c]
Grower					
ADG, kg	0.80	0.86	0.77	0.81	6.98[d]
Feed:gain	2.68	2.27	2.50	2.40	7.67[d]
Finisher					
ADG, kg	0.76	0.76	0.73	0.75	4.52
Feed:gain	3.72	3.71	3.65	3.67	2.18
Grower-finisher					
ADG, kg	0.78	0.80	0.75	0.79	2.78[b]
Feed:gain	3.24	3.05	3.16	3.12	3.93[e]
Total					
ADG, kg	0.66	0.63	0.62	0.62	3.05[f]
Feed:gain	2.90	2.81	2.93	2.91	3.67

[a]Protein effect $P<.01$.

[b]Level of feeding effect $P<.01$.

[c]Protein x level of feeding $P<.05$.

[d]Level of feeding effect $P<.05$.

[e]Level of feeding effect $P<.10$.

[f]Protein effect $P<.05$.

observed in this period. There were no treatment differences for average daily gain or feed:gain ratios during the finisher period. For the grower-finisher period, pigs that were previously restricted-fed gained weight faster ($P<.01$) and slightly more efficiently ($P<.10$) than did the full-fed pigs. For the entire feeding period, pigs that were fed the higher protein intake in the starter period gained weight faster ($P<.05$) than those that were fed the lower protein intake.

The summary of nitrogen balance studies is found in Table 4. During the last 5 days of the 44-day starter period, pigs that were fed the lower-protein intake retained nitrogen more efficiently ($P<.05$) than those that were fed the higher-protein intake, when expressed as percent of either nitrogen consumed or nitrogen absorbed. The pigs in these two groups had similar nitrogen retention expressed per kilogram of body weight$^{0.75}$ or per kilogram of body weight gain. Pigs that were restricted-fed retained more ($P<.05$) nitrogen per kilogram weight gain in the starter collection period than those that were full-fed. All pigs had similar nitrogen retention per 100 g of nitrogen consumed or absorbed during the 4th to 8th days of grower period. Pigs that were previously fed the lower-protein intake retained more ($P<.05$) nitrogen per kilogram body weight$^{0.75}$ than those that were in the high-protein groups. Similarly, previously restricted-fed pigs retained more ($P<.01$) nitrogen per kilogram body weight$^{0.75}$ when compared with the pigs that had been full-fed. Nitrogen retention per kilogram body weight gain was similar for all treatments in the grower period. During the early finisher period, pigs that were previously fed high-protein intake retained more ($P<.05$) nitrogen than the other group when expressed as grams per 100 g of nitrogen consumed

and as grams per kilogram body weight$^{0.75}$. No treatment differences were observed when nitrogen retention was expressed as a percentage of nitrogen absorbed.

Table 4. Experiment 7119 - Effect of starter nutrition on nitrogen balance during three 5-day collection periods

	Treatments				
	1	2	3	4	
Protein, %	18	27	12	18	C.V.
Level of feeding, %	100	66.7	100	66.7	%
Starter					
N retained, g:100 g N consumed	52.94	49.27	61.40	65.07	19.53[a]
N retained, g:100 g N absorbed	61.51	55.48	72.83	73.94	18.67[a]
N retained, g:kg body weight$^{0.75}$	8.23	8.77	6.90	6.31	22.61
N retained, g:kg body weight gained	36.79	59.89	30.98	55.95	41.15[b]
Grower					
N retained, g:100 g N consumed	50.07	50.63	51.26	52.26	7.91
N retained, g:100 g N absorbed	60.45	59.98	62.13	63.96	8.84
N retained, g:kg body weight$^{0.75}$	6.88	8.15	7.84	8.44	7.54[a,c]
N retained, g:kg body weight gained	24.43	31.02	27.65	28.09	17.13
Finisher					
N retained, g:100 g N consumed	44.47	39.21	34.74	38.55	12.27[a]
N retained, g:100 g N absorbed	57.52	49.93	46.28	50.29	11.83
N retained, g:kg body weight$^{0.75}$	3.81	3.60	2.88	3.44	12.85[a]
N retained, g:kg body weight gained	-	-	-	-	-

[a]Protein effect $P<.05$.

[b]Level of feeding effect $P<.05$.

[c]Level of feeding effect $P<.01$.

Summaries of plasma glucose, urea nitrogen and alpha-amino nitrogen during the early grower and finisher periods are given in Tables 5, 6 and 7. Plasma obtained during a fast in the early grower period contained more ($P<.01$) glucose, urea nitrogen and alpha-amino nitrogen than that in

the early finisher period. During both periods, there were no significant dietary treatment differences in plasma glucose and urea nitrogen; however, pigs that were previously restricted-fed had higher ($P<.05$) alpha-amino nitrogen content in plasma than those that were full-fed. Careful examination of Table 5 revealed that this treatment difference was primarily attributed to the difference during the early grower period. Plasma glucose did not change significantly with time of fast. Plasma urea nitrogen and alpha-amino nitrogen, however, decreased linearly with time of fasting ($P<.01$). Also, a significant ($P<.01$) quadratic effect of time of fasting on plasma urea nitrogen content was observed.

Table 5. Experiment 7119 - Effect of starter nutrition on plasma constituents during early grower and early finisher periods

	Treatments			
	1	2	3	4
Protein, %	18	27	12	18
Level of feeding, %	100	66.7	100	66.7
	Plasma glucose mg/100 ml[a]			
Grower	89.33	92.32	91.27	93.67
Finisher	85.31	87.86	87.27	83.53
	Plasma urea nitrogen mg/100 ml[a]			
Grower	12.90	11.87	12.13	12.20
Finisher	10.46	9.19	9.06	9.25
	Plasma α-amino nitrogen mM/100 ml[a,b]			
Grower	0.45	0.49	0.46	0.50
Finisher	0.40	0.41	0.38	0.40

[a]Grower *vs.* finisher $P<.01$.

[b]Level of feeding effect $P<.05$.

Table 6. Experiment 7119 - Effect of starter nutrition on plasma constituents at various times after fasting

	Treatments				
	1	2	3	4	
Protein, %	18	27	12	18	
Level of feeding, %	100	66.7	100	66.7	Average
Time, hr					
	Plasma glucose mg/100 ml				
0	84.53	90.24	90.74	84.36	87.47
2	93.67	92.64	81.96	96.13	91.10
4	85.19	91.90	92.60	94.05	90.94
8	89.64	88.78	92.00	85.12	88.73
16	83.55	86.88	89.06	83.93	85.85
Average	87.32	90.09	89.27	88.60	
	Plasma urea nitrogen mg/100 ml[a,b]				
0	12.11	10.99	10.52	10.68	11.07
2	13.03	11.71	11.34	11.68	11.94
4	12.71	12.17	12.07	12.49	12.36
8	11.11	9.77	10.60	10.74	10.56
16	9.43	8.00	8.45	8.04	8.48
Average	11.68	10.53	10.59	10.73	
	Plasma α-amino nitrogen mM/100 ml[a]				
0	0.48	0.51	0.47	0.49	0.49
2	0.48	0.50	0.46	0.53	0.49
4	0.42	0.45	0.43	0.45	0.44
8	0.40	0.40	0.38	0.40	0.39
16	0.36	0.37	0.36	0.38	0.37
Average	0.43	0.45	0.42	0.45	

[a]Linear time effect $P<.01$.

[b]Quadratic time effect $P<.01$.

Table 7. Experiment 7119 - Effect of time after fasting on plasma constituents during growing and finishing periods

	Time after fasting (hr)				
	0	2	4	8	16
	Plasma glucose mg/100 ml[a]				
Grower	90.73	97.49	96.82	88.04	85.16
Finisher	84.15	84.17	84.59	89.97	87.45
	Plasma urea nitrogen mg/100 ml[a,b,c]				
Grower	11.61	13.41	14.42	12.36	9.57
Finisher	10.67	10.45	10.19	8.69	7.47
	Plasma α-amino nitrogen mM/100 ml[a,b]				
Grower	0.49	0.58	0.48	0.42	0.39
Finisher	0.49	0.40	0.39	0.36	0.34

[a]Grower vs. finisher P<.01.

[b]Linear time effect P<.01.

[c]Quadratic time effect P<.01.

Summaries of chemical composition in three body components (carcass, offal and empty body) are given in Tables 8, 9 and 10. There were no significant treatment differences in water, protein, ether extract or ash contents of these body components at 93 kg live weight. However, the pigs that had been restricted-fed had slightly less (P<.10) ether extract in the empty body than that of the full-fed pigs. When these chemical components were adjusted by covariance to an equal empty body weight (Table 10), the restricted-fed pigs had significantly (P<.05) less ether extract in the carcass and empty body than did the full-fed groups. Significant (P<.05) feeding level x protein interactions were also observed for the protein and ash contents of offal samples. Restriction of feed intake at

the higher protein intake increased protein and ash content of the offal, but the restriction of feed at the lower protein intake resulted in the decreases of these components.

Table 8. Experiment 7119 - Effect of starter nutrition on chemical body composition at 92.7 kg body weight

	Treatments				
	1	2	3	4	
Protein, %	18	27	12	18	C.V.
Level of feeding, %	100	66.7	100	66.7	%
Carcass					
Water, %	48.38	49.84	48.85	48.78	3.52
Protein, %	15.25	15.90	15.48	15.34	4.64
Ether extract, %	33.04	30.50	32.45	32.33	7.94
Ash, %	3.10	3.05	3.04	3.20	7.68
Offal					
Water, %	61.37	60.66	60.21	61.34	4.59
Protein, %	12.26	12.15	12.01	12.33	5.06
Ether extract, %	24.82	26.26	26.38	24.42	13.44
Ash, %	0.97	0.95	0.95	0.95	5.11
Empty body					
Water, %	49.84	51.20	50.31	50.30	3.10
Protein, %	14.91	15.43	15.04	14.98	4.38
Ether extract, %	32.13	29.96	31.68	31.37	7.18
Ash, %	2.86	2.79	2.77	2.94	7.95

Table 9. Experiment 7119 - Effect of starter nutrition on chemical body composition at 92.7 kg body weight

	Treatments				
	1	2	3	4	
Protein, %	18	27	12	18	C.V.
Level of feeding, %	100	66.7	100	66.7	%
Carcass					
Water, kg	35.96	35.96	34.76	36.80	5.81
Protein, kg	11.33	11.48	11.02	11.51	7.28
Ether extract, kg	24.59	21.99	23.20	24.26	9.53
Ash, kg	2.31	2.20	2.17	2.41	11.53
Offal					
Water, kg	5.83	6.25	6.31	6.21	6.89
Protein, kg	1.16	1.25	1.26	1.24	6.79
Ether extract, kg	2.37	2.69	2.79	2.48	14.92
Ash, kg	0.09	0.10	0.10	0.10	7.69
Empty body					
Water, kg	41.79	42.21	41.07	42.79	5.36
Protein, kg	12.50	12.73	12.29	12.75	6.95
Ether extract, kg	26.96	24.68	26.00	26.74	8.99
Ash, kg	2.40	2.30	2.27	2.51	11.21

Table 10. Experiment 7119 - Effect of starter nutrition on chemical body composition when adjusted to an equal empty body weight (82.9 kg)

	Treatments				
	1	2	3	4	
Protein, %	18	27	12	18	C.V.
Level of feeding, %	100	66.7	100	66.7	%
Carcass					
Water, kg	35.66	36.25	35.31	35.77	1.81
Protein, kg	11.22	11.59	11.23	11.21	3.54
Ether extract, kg	24.28	22.28	23.76	23.44	4.04[a]
Ash, kg	2.27	2.23	2.23	2.32	6.54
Offal					
Water, kg	5.80	6.27	6.35	6.14	6.68
Protein, kg	1.16	1.26	1.28	1.22	5.23[b]
Ether extract, kg	2.37	2.70	2.80	2.47	15.68
Ash, kg	0.09	0.10	0.10	0.09	5.93[b]
Empty body					
Water, kg	41.47	42.52	41.67	41.91	1.62[c]
Protein, kg	12.38	12.85	12.50	12.43	3.15
Ether extract, kg	26.65	24.98	26.56	25.91	3.56[a]
Ash, kg	2.36	2.33	2.33	2.41	6.20

[a]Level of feeding effect $P<.05$.

[b]Protein x feeding $P<.05$.

[c]Level of feeding effect $P<.10$.

The results presented in Table 11 are the summaries of muscle fiber diameter of L. dorsi at market weight. There were no dietary treatment differences with respect to muscle fiber diameter.

Nucleic acid and protein concentrations and relationships in the L. dorsi are summarized in Table 12. There were no significant differences in the concentrations of DNA, RNA and protein or in RNA:DNA and protein: DNA ratios in the L. dorsi samples at market weight.

Table 11. Experiment 7119 - Summary effect of starter nutrition on muscle fiber diameter of L. dorsi, microns

		Treatments				
		1	2	3	4	
Protein, %		18	27	12	18	C.V.
Level of feeding, %		100	66.7	100	66.7	%
Replications	1	71.1	70.7	71.9	75.6	
	2	64.7	70.8	75.0	78.3	
	3	71.7	73.9	74.2	79.7	
	4	69.7	81.4	71.1	64.5	
	5	74.8	63.1	64.5	71.9	
Average		70.4	72.0	71.3	74.0	8.40

Table 12. Experiment 7119 - Effect of starter nutrition on nucleic acid and protein concentrations and relationships in L. dorsi muscle of market pigs

	Treatments				
	1	2	3	4	
Protein, %	18	27	12	18	C.V.
Level of feeding, %	100	66.7	100	66.7	%
DNA, mg/g	0.332	0.329	0.304	0.328	11.36
RNA, mg/g	0.768	0.791	0.763	0.804	7.80
Protein, mg/g	191.68	197.71	194.79	191.02	8.30
RNA:DNA	2.32	2.42	2.51	2.49	9.10
Protein:DNA	580.50	611.03	646.13	600.00	16.89

Experiment 7215

Effect of Protein Nutrition on Cellularity and Body Composition of Starter Pigs

Objectives

Protein restriction in rats is thought to interrupt cell growth by reducing net protein synthesis (Cheek and Hill, 1970). The belief is supported by findings of Hill *et al.* (1970) who indicated that skeletal muscle of rats fed a restricted-protein diet had lower RNA and protein concentrations, RNA:DNA and protein:DNA ratios, but a higher DNA concentration when compared to muscle of unrestricted rats. Also, Moss (1968a) indicated that muscle weight, nuclei number and the fiber cross-sectional area of the *pectoral* and the *gastrocnemius* muscles of normally fed chicks increased in logarithmic proportion to each other. An 8-day feed restriction that retarded growth of the muscle to 70 percent of the controls did not change the logarithmic ratio of the fiber cross-sectional area:nuclei number but lowered the nuclei number and cross-sectional area per unit of muscle weight (Moss, 1968b). Because little work of a similar type has been done in baby pigs, the purposes of this experiment were: 1) to determine the effect of protein intake on performance, chemical composition and cellularity of starter pigs; 2) to determine the relationship between lean body mass and cell development in starter pigs; 3) to determine the effect of nutrition on cellularity of adipocytes; and 4) to determine the effect of variation of protein intake on muscle mass, fiber diameter, protein, nucleic acids and their relationships in the *Rectus femoris* of pigs fed to attain equivalent lean masses.

Procedure

Twenty early-weaned pigs averaging 5.0 kg body weight and 23 days of age were used in a randomized complete block design. There were five replications of four dietary treatment combinations. Four littermates were allotted at random to one of these treatment combinations: pigs in Treatments 1 and 2 were fed a 10 and a 20 percent crude protein, corn-soybean meal starter diets at the 100 percent full-feed level of the feeding scale previously established (Khajarern, 1971), for 50 days; pigs in Treatments 3 and 4 were fed the 10 and 20 percent protein diets at 100 percent full-feed level until they attained the estimated equal lean mass of pigs of Treatments 2 and 1, respectively. The total amount of diet used to promote equal lean mass was estimated by the equation: average daily lean mass gain (kg) = 0.11 + 1.1 (average daily protein intake, kg). The linear relationship between gain and intake was calculated from daily protein intake and water and protein gains of pigs in previous experiments (Wyllie et al., 1969; Zimmerman and Khajarern, 1973). Pigs were individually fed. The starter diets used were iso-caloric and equal in protein quality. Composition and calculated analyses of the diets are given in Tables 24 and 25.

One pig died during the course of the experiment. Cause of death was suspected to be porcine stress syndrome (PSS), a syndrome unrelated to the treatment. Missing values were calculated according to the method described by Snedecor and Cochran (1967).

The pigs were slaughtered at the end of the starter period. The left half of the carcass and the evacuated viscera plus blood were used for determination of moisture, total nitrogen, ether extract,

ash and nucleic acids. Sample preparation for these purposes was previously described.

The intact R. femoris from the right leg was dissected. Determinations of muscle weight, fiber diameter, nucleic acids, moisture, ether extract and protein concentration were made. Adipose cell number and lipid content in the superficial subcutaneous adipose tissue from the area of 10th to 12th rib were determined.

Special notes

Treatment effects must be interpreted in light of age effects on the parameters measured. Effects of protein and lean mass were confounded by age differences. Pigs of Treatment 3 (10 percent protein diet) reached an estimated equal lean mass at a much older age (89 days) than those of Treatment 2 (20 percent protein diet to 73 days of age). On the other hand, pigs of Treatment 4 (20 percent protein diet) reached the estimated lean mass at a much younger age (62 days) than pigs of Treatment 1 (10 percent protein diet to 73 days of age). If treated as a factorial arrangement, the main effect of protein intake or lean mass would not be independent from the effects of age. The average final age of pigs of the 20 percent protein treatments was 67.5 days and was 81 days for pigs of 10 percent protein treatments. Similarly, the average age of pigs fed to attain the higher lean mass was 81 days, whereas that for pigs fed to the lower lean mass was 67.5 days. Only the comparisons of Treatments 1 vs. 2 estimated the effect of protein intake independent of age effect. Comparisons of Treatments 1 vs. 4 and 2 vs. 3 estimated the effect of differential rates of lean mass gain but, by design, are confounded by

an age effect. Comparison of Treatments (1 + 4) vs. (2 + 3) estimated differences between the two levels of lean mass and are also confounded by an age effect.

Another source of variation that affects the results and the interpretation is that the amounts of feed to promote equal lean mass of Treatments 3 to 2 and 4 to 1 were not estimated perfectly. Body analysis revealed that pigs fed the 20 percent protein diet had more lean mass than their mates that were fed the 10 percent protein diet to a projected equal lean mass.

Results

Summaries of average daily gain and feed:gain ratio are given in Table 13. Pigs fed the 20 percent protein diet gained weight faster ($P<.01$) and had a smaller ($P<.01$) feed:gain ratio than did pigs fed the 10 percent protein diet (1 vs. 2) for the same length of time. Pigs fed to gain lean mass more rapidly (2 and 4) gained weight faster ($P<.01$) and utilized feed more efficiently ($P<.01$) than did pigs fed to gain lean mass slowly (1 and 3). Pigs fed to a higher lean mass (2 and 3) gained weight faster ($P<.01$) and utilized feed less efficiently ($P<.01$) than did pigs fed to attain a lower lean mass (1 and 4). This difference expresses an age effect.

Effect of early nutrition on chemical body composition of pigs at the end of the starter period are summarized in Tables 14 and 15. Pigs fed the 20 percent protein diet had more ($P<.01$) total water, protein and ash but less ($P<.01$) fat in the carcass and empty body than did pigs fed the 10 percent protein diet to the same age (1 vs. 2). Similar treatment

Table 13. Experiment 7215 - Effect of early nutrition on the average daily gain and feed:gain ratio of pigs during starter period

	Treatments				
	1	2	3	4	
Protein, %	10	20	10	20	C.V.
Feeding regime	Lean I	Lean II	Lean II	Lean I	%
Average daily gain, kg	0.28	0.39	0.31	0.38	3.84[a,b,c,d]
Feed:gain	2.60	1.89	2.79	1.63	5.53[a,b,c,d]

[a]Treatments 1 vs. 2 P<.01.

[b]Treatments 1 vs. 4 P<.01.

[c]Treatments 2 vs. 3 P<.01.

[d]Treatments 1 + 4 vs. 2 + 3 P<.01.

Table 14. Experiment 7215 - Effect of early nutrition on chemical body composition at the end of starter period

	Treatments 1	2	3	4	
Protein, %	10	20	10	20	C.V.
Feeding regime	Lean I	Lean II	Lean II	Lean I	%
Carcass weight, kg	15.17	19.34	19.26	14.11	
Water, kg	8.65	12.73	10.66	9.71	5.62[a,b,c,d]
Protein, kg	2.23	3.40	2.82	2.44	5.48[a,b,c,d]
Ether extract, kg	3.76	2.59	5.16	1.40	4.45[a,b,c,d]
Ash, kg	0.56	0.65	0.69	0.50	6.54[a,b,c,d]
Offal weight, kg	3.29	4.28	4.77	3.97	
Water, kg	2.58	3.37	3.72	3.21	7.45[a,e,f]
Protein, kg	0.46	0.67	0.66	0.60	5.13[a,d,e]
Ether extract, kg	0.19	0.17	0.30	0.10	1.67[c,d,e]
Ash, kg	0.034	0.046	0.046	0.043	7.14[a,d,e]
Empty body weight, kg	18.46	23.62	24.03	18.08	
Water, kg	11.24	16.11	14.39	12.92	5.11[a,c,d,e]
Protein, kg	2.69	4.07	3.48	3.04	4.64[a,c,d,e]
Ether extract, kg	3.95	2.76	5.46	1.50	4.23[a,c,d,e]
Ash, kg	0.59	0.70	0.74	0.54	6.19[a,d]

[a]Treatments 1 vs. 2 P<.01.

[b]Treatments 1 vs. 4 P<.05.

[c]Treatments 2 vs. 3 P<.01.

[d]Treatments 1 + 4 vs. 2 + 3 P<.01.

[e]Treatments 1 vs. 4 P<.01.

[f]Treatments 2 vs. 3 P<.05.

Table 15. Experiment 7215 - Effect of early nutrition on chemical body composition at the end of starter period

	Treatments				
	1	2	3	4	
Protein, %	10	20	10	20	C.V.
Feeding regime	Lean I	Lean II	Lean II	Lean I	%
Carcass					
Water, %	57.04	65.78	55.32	68.86	1.53[a,b,c,d]
Protein, %	14.70	17.62	14.64	17.30	2.68[a,b,c]
Ether extract, %	24.75	13.42	26.86	18.75	5.38[a,b,c,d]
Ash, %	3.69	3.39	3.60	3.52	7.78
Offal					
Water, %	78.57	78.92	78.16	80.72	1.34[b,e]
Protein, %	13.95	15.60	13.90	15.03	3.33[a,b,c]
Ether extract, %	5.79	3.98	6.32	2.40	18.95[a,b,c,e]
Ash, %	1.03	1.08	0.97	1.09	4.81[c]
Empty body					
Water, %	60.87	68.17	59.84	71.48	1.20[a,b,c,d]
Protein, %	14.56	17.25	14.49	16.80	2.43[a,b,c]
Ether extract, %	21.38	11.71	22.79	8.27	5.22[a,b,c,d]
Ash, %	3.22	2.97	3.08	2.98	6.78

[a]Treatments 1 vs. 2 P<.01.

[b]Treatments 1 vs. 4 P<.01.

[c]Treatments 2 vs. 3 P<.01.

[d]Treatments 1 + 4 vs. 2 + 3 P<.01.

[e]Treatments 1 + 4 vs. 2 + 3 P<.05.

differences were observed for the offal, except that there was no difference in total amount of offal fat. Pigs fed to have faster rates of lean mass gain (2 and 4) had more (P<.01) total water and protein, but less (P<.01) fat in the empty body than did pigs fed to gain lean mass at a slower rate (1 and 3). No significant differences between pigs of these two groups were found in total empty body ash. Pigs fed to attain the higher

lean mass (2 + 3) had more (P<.01) total water, protein, fat and ash in the empty body than did pigs fed to a lower lean mass (1 + 4). When each chemical component in the empty body was expressed as a percentage (Table 15), similar treatment differences, as seen in total amounts, were observed except that the ash concentration was the same for all treatments. In addition, the protein concentration of pigs in the two lean mass groups (1 + 4 vs. 2 + 3) was not significantly different.

Table 16 presents the summaries of treatment effects on nucleic acids and protein in the empty body. Pigs fed the 20 percent protein diet had lower concentrations of DNA (P<.05) and RNA (P<.05), but higher concentrations of protein (P<.01), more total protein (P<.01), faster rates of protein gain (P<.01) and wider protein:DNA ratios (P<.01) in the empty body than did pigs of the same age fed the 10 ercent protein diet (1 vs. 2). The total empty body DNA and RNA, DNA and RNA daily gains, and RNA:DNA ratio of pigs in these two treatments were not significantly different. Pigs fed to have a faster rate of lean mass gain (2 and 4) had higher (P<.01) protein concentrations, more (P<.01) total empty body protein and higher (P<.01) daily protein deposition in the body when compared with pigs fed to have a slower rate of lean gain (1 and 3). Total empty body DNA and RNA, their concentrations and relationships in the empty body of pigs in these groups (2 vs. 3 and 1 vs. 4) were not significantly different. Pigs fed to attain the higher lean mass (2 and 3) had lower concentrations of DNA (P<.01), RNA (P<.05) and daily DNA gain (P<.05), but higher (P<.01) total empty body protein, daily protein gain and wider (P<.01) protein:DNA ratios than those fed to attain the lower lean mass (1 and 4). Age differences probably account for some of these differences.

Table 16. Experiment 7215 - Effect of early nutrition on the nucleic acid and protein content of empty body

	Treatments				
	1	2	3	4	
Protein, %	10	20	10	20	C.V.
Feeding regime	Lean I	Lean II	Lean II	Lean I	%
DNA concentration, mg/g	1.28	0.93	0.96	1.18	16.36[a,b]
Total, g	23.56	21.88	22.86	21.50	16.10
Gains, g/day	0.27	0.24	0.20	0.30	26.58[a]
RNA concentration, mg/g	0.76	0.55	0.67	0.75	16.57[a,c]
Total, g	14.00	12.96	15.86	13.72	17.10
Gains, g/day	0.18	0.16	0.18	0.23	27.81
Protein concentration, mg/g	145.64	172.50	144.94	160.02	2.43[d,e,f]
Total, kg	2.69	4.07	3.48	3.04	4.64[b,d,e,f]
Gains, g/day	41.05	68.92	43.20	61.79	4.72[b,d,e,f]
RNA:DNA	0.62	0.62	0.72	0.66	21.67
Protein:DNA	118.66	189.82	163.15	145.48	16.79[b,d]

[a] Treatments 1 vs. 2 P<.05.

[b] Treatments 1 + 4 vs. 2 + 3 P<.01.

[c] Treatments 1 + 4 vs. 2 + 3 P<.05.

[d] Treatments 1 vs. 2 P<.01.

[e] Treatments 1 vs. 4 P<.01.

[f] Treatments 2 vs. 3 P<.01.

Summaries of the effect of nutrition on the R. femoris are given in Tables 17 and 18. Data in Table 17 indicated that pigs fed the 20 percent protein diet (2) had heavier whole muscle weight ($P<.01$) and weight per nucleus ($P<.05$), faster ($P<.01$) daily muscle weight gain, faster ($P<.05$) increase in fiber diameter per day, higher ($P<.05$) water concentration but lower ($P<.01$) fat concentration than did pigs fed the 10 percent protein diet (1) for the same length of time. Muscle fiber diameter and muscle protein concentration of pigs of these two treatments, however, were similar. Pigs fed to have a faster rate of lean mass gain had heavier (4 vs. 1, $P<.05$; 2 vs. 3, $P<.01$) R. femoris, faster ($P<.01$) rates of muscle weight gain and diameter expansion, and higher ($P<.01$) water concentration than did pigs that gained lean mass at a slower rate. Fat concentration, however, was lower ($P<.01$) in the muscle of pigs fed to have the faster rate of lean gain. The R. femoris of pigs fed to higher lean mass (2 and 3) were heavier ($P<.01$), gained weight more rapidly ($P<.05$), had larger ($P<.05$) fiber diameter, weighed more ($P<.05$) per nucleus and had higher ($P<.05$) fat concentration than did muscle of the lower lean mass pigs (1 and 4). Again, these responses are affected by age differences.

Data in Table 18 indicated that pigs fed the 20 percent protein diet (2) had higher total R. femoris DNA ($P<.05$), daily DNA gain ($P<.05$), RNA ($P<.01$), daily RNA gain ($P<.01$), protein ($P<.01$), daily protein gain ($P<.01$) and a wider protein:DNA ratio ($P<.01$) than did pigs fed the 10 percent protein (1) to an equal age. However, there were lower concentrations of DNA ($P<.01$) and RNA ($P<.05$) in the muscle of pigs fed 20 percent protein than in muscle of those fed 10 percent protein (2 vs. 1). The

Table 17. Experiment 7215 - Effect of early nutrition on R. femoris muscle

	Treatments				
	1	2	3	4	
Protein, %	10	20	10	20	C.V.
Feeding regime	Lean I	Lean II	Lean II	Lean I	%
Muscle mass					
Weight, g	129.60	207.40	171.10	148.30	7.45[a,b,c,d]
Weight gain, g/day	1.96	3.53	2.10	3.02	8.97[a,c,e,f]
Weight:nucleus, $gx10^{-9}$/nucleus	14.15	17.63	16.51	15.27	7.44[f,g]
Muscle fiber					
Diameter, μ	43.60	48.00	47.00	44.00	7.49[f]
Diameter gain, μ/day	0.41	0.50	0.38	0.54	14.12[c,e,g]
Chemical composition					
Water, %	76.11	77.40	75.65	78.11	1.07[c,e,g]
Protein, %	19.21	20.20	19.81	19.94	4.23
Ether extract, %	4.34	2.21	4.83	1.86	10.84[c,e,f]

[a]Treatments 1 vs. 2 P<.01.

[b]Treatments 1 vs. 4 P<.05.

[c]Treatments 2 vs. 3 P<.01.

[d]Treatments 1 + 4 vs. 2 + 3 P<.01.

[e]Treatments 1 vs. 4 P<.01.

[f]Treatments 1 + 4 vs. 2 + 3 P<.05.

[g]Treatments 1 vs. 2 P<.05.

Table 18. Experiment 7215 - Effect of early nutrition on nucleic acid and protein content of R. femoris muscle

	Treatments				
	1	2	3	4	
Protein, %	10	20	10	20	C.V.
Feeding regime	Lean I	Lean II	Lean II	Lean I	%
DNA concentration, mg/g	0.45	0.35	0.38	0.41	12.61[a,b]
Total, mg	57.87	72.79	65.46	60.57	14.42[b,c]
Gain, mg/day	0.42	0.72	0.44	0.61	31.21[c,d]
RNA concentration, mg/g	1.19	1.08	1.00	1.30	6.86[c,e]
Total, mg	154.33	223.98	172.81	193.78	6.91[a,e,f,g]
Gain, mg/day	2.08	3.49	1.81	3.70	10.41[a,f,g]
Protein concentration, mg/g	192.14	201.96	198.08	199.40	4.23
Total, g	24.72	41.80	33.33	29.55	5.98[a,e,f,g]
Gain, mg/day	0.37	0.71	0.41	0.60	6.75[a,e,f,g]
RNA:DNA	2.78	3.77	2.61	3.22	15.64
Protein:DNA	483.79	573.96	518.67	492.22	12.77[a,b]
Lean weight, log of g[h]	2.11	2.31	2.22	2.16	
Nuclei number, log of 10^{9h}	9.97	10.07	10.04	9.99	
Fiber cross-sectional area, log of μ^{2h}	3.17	3.26	3.26	3.18	

[a]Treatments 1 vs. 2 P<.01.

[b]Treatments 1 + 4 vs. 2 + 3 P<.05.

[c]Treatments 1 vs. 2 P<.05.

[d]Treatments 2 vs. 3 P<.05.

[e]Treatments 1 + 4 vs. 2 + 3 P<.01.

[f]Treatments 2 vs. 3 P<.01.

[g]Treatments 1 vs. 4 P<.01.

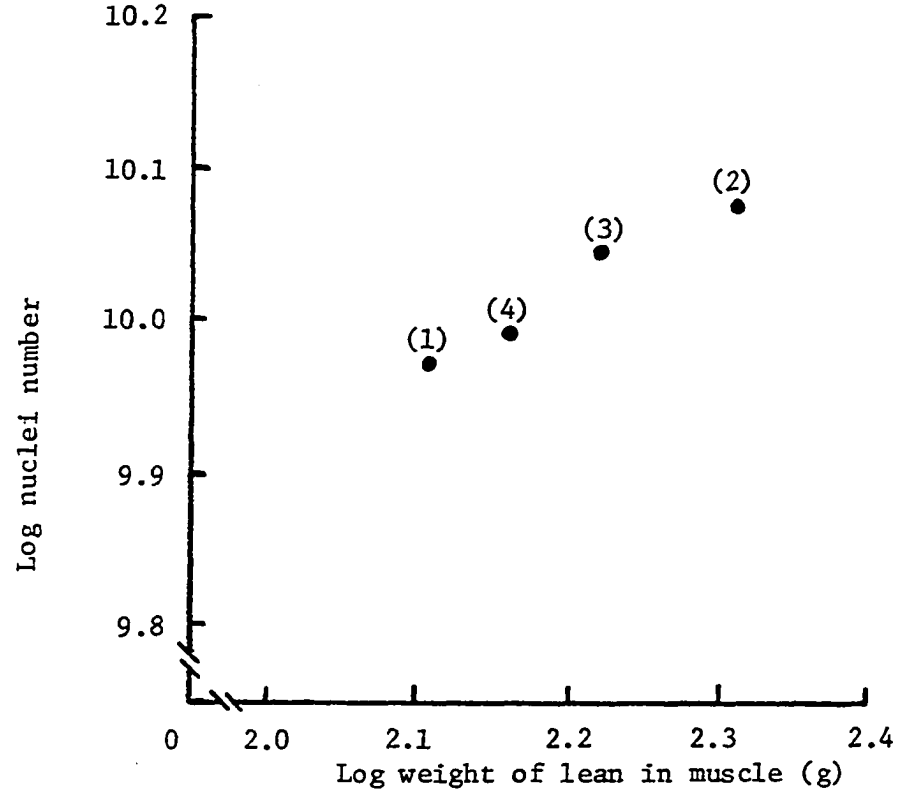
[h]For use in relationship plots, see Figures 1, 2 and 3.

muscle protein concentration and RNA:DNA ratio were similar for pigs of both groups. Pigs that were fed to have a faster rate of lean mass gain had faster rates of DNA (1 vs. 4, P<.01; 2 vs. 3, P<.05), RNA (P<.01) and protein (P<.01) gains than did pigs fed to have a slower rate of lean mass gain. Similarly, the R. femoris of the pigs depositing lean rapidly contained more (P<.01) RNA and protein than did the others. No significant differences between muscles of these two groups were observed with respect to concentrations of DNA, RNA and protein, total DNA, RNA:DNA ratio and protein:DNA ratio. The R. femoris of the high-lean-mass pigs (2 and 3) had more total DNA (P<.05), RNA (P<.01), faster daily DNA gain (P<.05), faster daily protein gain (P<.01) and a wider protein:DNA ratio (P<.05) than did the muscle of pigs of the low-lean-mass groups (1 and 4). The reverse was true for the muscle DNA and RNA concentrations, while no significant differences were observed for protein concentrations and RNA:DNA ratios in pigs of these two groups.

Logarithmic values of weight of lean (protein + water), nuclei number and the fiber cross-sectional area of the R. femoris are listed in the last part of Table 18 and their relationships are plotted in Figures 1, 2 and 3. In a chick muscle that is growing in length and width, the relationships of pairs of muscle weight and nuclei number, muscle weight and the fiber cross-sectional area, and nuclei number and the fiber cross-sectional area are linear and not affected by age differences (Moss, 1968a). In the present study, weight of lean rather than total muscle weight was used because treatments greatly affected the lipid concentration of the R. femoris. Protein plus water in muscle is a better estimate of muscle fiber mass than is total muscle weight.

Figure 1. Experiment 7215 - Relationship between nuclei number and weight of lean in the R. femoris

Figure 2. Experiment 7215 - Relationship between the fiber cross-sectional area and weight of lean in the R. femoris


10.2
10.1
10.0
9.9
9.8
Log nuclei number
(1)
(2)
(3)
(4)
0
2.0
2.1
2.2
2.3
2.4
Log weight of lean in muscle (g)

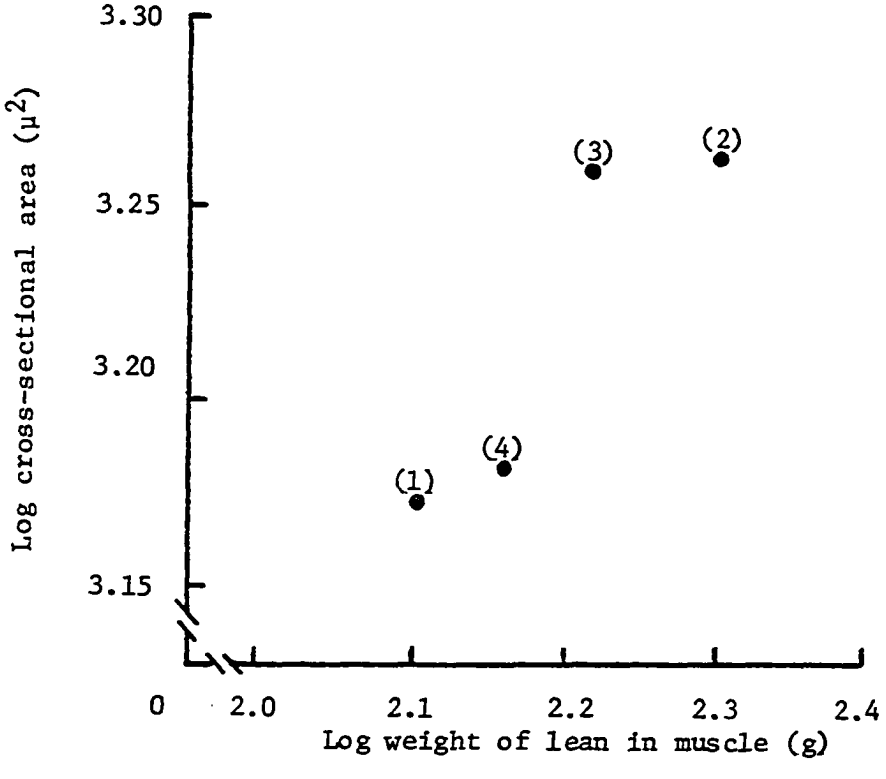
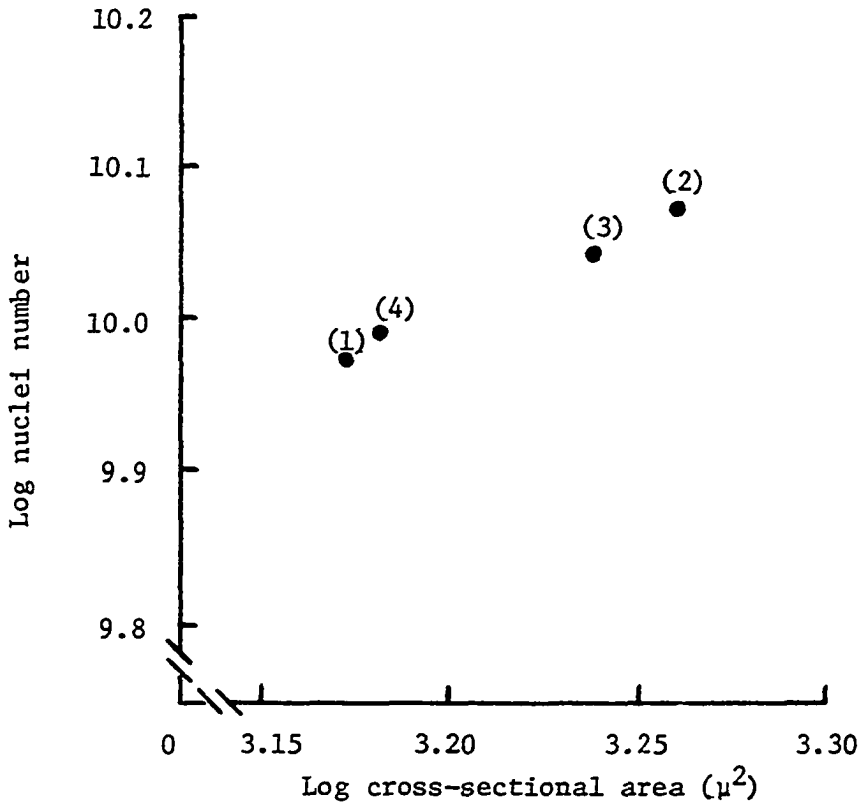

3.30
3.25
3.20
3.15
Log cross-sectional area (μ²)
(1)
(2)
(3)
(4)
0
2.0
2.1
2.2
2.3
2.4
Log weight of lean in muscle (g)

Figure 3. Experiment 7215 – Relationship between nuclei number and cross-sectional area of the R. femoris muscle fiber


10.2
10.1
10.0
9.9
9.8
Log nuclei number
(1)
(2)
(3)
(4)
0
3.15
3.20
3.25
3.30
Log cross-sectional area (μ²)

Because of the insufficient degrees of freedom, statistical comparisons of the linear regression slopes in Figures 1, 2 and 3 were not made. Consequently, the inferences to be made were based entirely on gross comparisons. Figure 1 shows that the straight line that passes through means of Treatments 1 and 3 is higher than that for Treatments 2 and 4. This indicates that the R. femoris nuclei number per gram of the muscle lean weight is higher for pigs that gain lean mass at a slower rate than the others. However, this might or might not be a real difference. The fiber cross-sectional area per unit of the muscle lean mass (Figure 2) was higher for pigs of Treatments 1 and 3 than those of Treatments 2 and 4. This indicated shorter muscle fibers in pigs that gain lean mass at a slower rate than that of pigs in the other group. Figure 3 shows a rather linear relationship between nuclei number and cross-sectional area of the muscle fiber. Rate of lean mass gain did not seem to affect the relationship between these two criteria.

Table 19 presents the summaries of the effect of treatments on cellularity of adipocytes in back fat. The adipocyte number per unit of tissue weight or number relative to age was not affected by treatments. Lipid content per cell and per unit of adipose tissue weight, however, decreased ($P<.05$ and $P<.01$, respectively) with increased rate of lean mass gain. Lipid content per cell relative to age was not affected by treatments. Lipid concentration in adipose tissue increased ($P<.01$) with increase of lean mass, but this increase was also associated with an increase in average age (67.5 vs. 81 days).

Table 19. Experiment 7215 - Effect of nutrition on adipocyte cellularity and lipid content of subcutaneous adipose tissue

	Treatments				
	1	2	3	4	
Protein, %	10	20	10	20	C.V.
Feeding regime	Lean I	Lean II	Lean II	Lean I	%
Cell numbers					
Cells/mg tissue, ($x10^3$)	4.31	4.18	4.89	4.00	11.78
Cells/mg/day of age	59.36	57.63	54.97	65.59	13.04
Cell size					
µmoles ester/cell, ($x10^{-4}$)	6.12	5.68	6.83	5.11	12.05[a,b]
µmoles ester/cell/day of age, ($x10^{-6}$)	8.43	7.90	7.73	8.31	11.59
Lipid concentration					
µmoles ester/mg tissue	2.55	2.31	3.60	1.97	9.59[c,d,e]

[a]Treatments 1 vs. 4 P<.05.

[b]Treatments 2 vs. 3 P<.05.

[c]Treatments 1 vs. 4 P<.01.

[d]Treatments 2 vs. 3 P<.01.

[e]Treatments 1 + 4 vs. 2 + 3 P<.01.

GENERAL DISCUSSION

Performance and Body Composition

Rate and efficiency of body weight gains of pigs during the starter period can be altered by nutrition in the period. In Experiment 7119, restricted-fed pigs gained weight slower but more efficiently than unrestricted pigs. Similar findings for rate of weight gain have been reported by Bowland and Berg (1959) and Frape et al. (1959). Rousselow (1973) and Zimmerman and Khajarern (1973) observed improvement in feed:gain ratio of starter pigs being fed restrictively.

Compensatory growth response is evident in Experiment 7119. Pigs that were previously restricted-fed gained weight faster and more efficiently than unrestricted pigs during the grower period. Concomitantly, apparent nitrogen retained per kilogram of physiological body size and circulating levels of alpha-amino nitrogen in plasma during a fast were higher in restricted than in unrestricted pigs. These responses may indicate more active protein deposition and amino acid transport in previously restricted than unrestricted pigs during the grower period. No significant differences between the two feeding levels were observed with respect to rate of weight gain, feed efficiency and nitrogen retention during the finisher period. This indicated that compensatory growth responding to feed restriction was completed before the finisher period. In addition, market pigs that were previously restricted-fed had slightly more water and less fat in the body than those in the unrestricted groups, when the data were adjusted to an equal empty body weight. Increased rate of weight gain, improved feed:gain ratio and

greater carcass leanness at market weight of previously restricted-fed pigs were reported by Elsley (1963), Nielson (1964), Duckworth (1965) and Vanschoubrock et al. (1965). Zimmerman and Khajarern (1973) reported that previously restricted-fed pigs deposited more protein into the body than did unrestricted pigs; however, no treatment differences were observed between these two groups for rate and efficiency of gains during the rehabilitation period. Several workers (Frape et al., 1959; Boaz and Elsley, 1962; Rousselow, 1973) failed to detect alterations of subsequent rate or efficiency of weight gains or of final chemical body composition in response to early feeding levels.

Results from Experiments 7119 and 7215 showed that pigs fed low-protein diets gained slower and less efficiently than those fed high-protein diets during the starter period. These findings have been repeatedly demonstrated by several workers (Meade et al., 1969b; Wyllie et al., 1969; Rousselow, 1973; Zimmerman and Khajarern, 1973). In addition, chemical body composition of pigs at the end of the starter period can be easily manipulated by protein levels in the diets (Experiment 7215; Wyllie et al., 1969; Zimmerman and Khajarern, 1973). In Experiment 7215 pigs fed to have a faster rate of lean mass gain grew faster, utilized feed more efficiently and had less fat in the empty body than did pigs fed to have a slower rate of lean mass gain. A larger lean mass was associated with faster average daily gain, larger feed:gain ratio and more body fat content. These results confirmed the findings of Zimmerman and Khajarern (1973).

Compensatory weight gain and feed:gain ratio responding to starter protein intake were somewhat delayed and less pronounced when compared

with those responding to energy intake. In neither Experiment 7119, nor the report of Zimmerman and Khajarern (1973), have significant differences between pigs fed two starter protein intakes been observed for rate or efficiency of grower weight gains or for chemical composition of market pigs. During the finisher period, no treatment differences in rate or efficiency of weight gains were observed in Experiment 7119, while improved feed:gain ratio of low-protein-fed pigs was demonstrated by Zimmerman and Khajarern (1973).

Results from the nitrogen balance studies (Experiment 7119) do not indicate delayed compensatory response. Nitrogen retention by low-protein pigs was more in the grower period, but less in the finisher period, than that by high-protein pigs. There have been very few reports on nitrogen balance of pigs during a compensatory period. Rousselow (1973) found no differences in nitrogen retention by pigs being switched from diets containing 10 to 31 percent protein to a common 16 percent protein diet. In rats, Barnes et al. (1973) observed an increase, while Lee and Chow (1965, 1968) observed a decrease in nitrogen retention by previously malnourished rats when compared with controls. A slower protein catabolism of previously low-protein-fed animals, when compared with high-protein groups, has been suggested by Baur and Filer (1959) and Vaughan et al. (1962) in pigs, by Chan (1968) in human and by Japanese workers in rats (Nakano and Ashida, 1970; Nakano et al., 1972).

Cellular Responses

There are a limited number of reports concerning the effect of early nutrition on cellularity of muscle, adipose tissue and the whole body.

Most of these are reports of rat and human studies. Since these species are known to be different from pigs in physiological maturity at birth, the cellular responses may or may not be similar.

In Experiment 7215 pigs fed the diet higher in protein content had heavier R. femoris and slightly larger (1 vs. 2, P<.10) fiber diameter, at the end of the starter period, than did pigs fed the lower-protein diet. Daily muscle weight gain and diameter expansion were also higher in the pigs fed the higher-, than in the pigs fed the lower-protein levels. These results were in agreement with that of Staun (1963) who found that pigs fed diets increasing in protein content, up to a limit, had larger muscle fiber diameter than those fed diets containing lower-protein level to the same age. Increasing lean mass of pigs in Experiment 7215 was associated with increases in muscle weight and fiber diameter. A result similar to this observation was reported by Chrystall and Zobrisky (1967) who demonstrated that muscle fiber diameter of pigs increased with age and body weight to at least 150 days of age.

Skeletal muscle of postweaning pigs grow by a combination of hyperplasia and hypertrophy. Hyperplasia and hypertrophy of the R. femoris of starter pigs were evident in the present study. Nucleus multiplication (increase in muscle DNA) and cytoplasmic growth (increase in weight: nucleus or protein:DNA) were associated with increased protein intake. Pigs fed the 20 percent protein diets had more nuclei and larger fibers than did the muscle of pigs fed the 10 percent protein diet to the same age. Similar effects of protein intake were also observed for RNA, protein and for the daily gain of DNA, RNA and protein in the muscle. RNA:DNA ratios, however, were not significantly different. Concentrations

of muscle DNA and RNA decreased as the protein intake increased. These decreases, along with the increased protein concentration, indicated a higher increase of muscle protein per nucleus of the high-, than of the low-protein-fed pigs. Results similar to the findings of Experiment 7215 were reported by Strunz and Lenkeit (1964) and Strunz et al. (1966) in pigs, Mendes and Waterlow (1958) and Dickerson et al. (1972) in rats and Robinson and Lambourne (1970) in mice. Also, Moser et al. (1972) noted an indication of larger muscle cells (lower DNA:muscle weight) in the gracilis of high-protein, than that of low-protein-fed pigs at the same age. Cheek and Hill (1970) hypothesized that protein intake primarily regulated cytoplasmic growth, whereas, energy intake regulated nucleus multiplication in rat muscle. Energy intake was not a limiting factor in Experiment 7215; consequently, only protein intake influenced the cellular responses. Protein:DNA ratio increased with the increase of protein intake and hence followed the hypothesis. Nucleus multiplication (DNA gain per day) also increased with the increased protein intake. This observation is not readily explained by the hypothesis. Activity of the replicating system might be affected by protein intake.

In chicks fed a normal diet, Moss (1968a) demonstrated linear relationships between nuclei number and muscle weight, nuclei number and cross-sectional area of muscle fibers and muscle weight and cross-sectional area of the fibers in the pectoral muscle when all parameters were in logarithmic scale. In chicks growing normally the fiber cross-sectional area and nuclei number increased in direct proportion; muscle weight increased at two-thirds of their rate. It follows that muscle fiber volume increases at two-thirds of the rate of increase of nuclei number. He also

indicated that the length and diameter of the fibers maintain a constant ratio until the muscle reaches a certain length. Subsequently, Moss (1968b) demonstrated that a level of feed restriction from 8 to 16 days, that retarded growth of the muscle to 70 percent of the controls, did not affect the relationship between number of nuclei and the fiber cross-sectional area but disrupted the relationship of these two criteria to the weight of the muscle. The logarithmic nuclei number:muscle weight and cross-sectional area:muscle weight ratios were lowered by the restriction. The ad libitum refeeding caused compensatory growth and restored the relationships.

Based on the information provided by the reports of Moss (1968a,b) and Cheek and Hill (1970), a working hypothesis to explain compensatory lean mass growth is proposed. Pigs fed a restricted protein diet (slower rate of lean gain) develop more nuclei per unit of lean mass than those fed a higher protein intake (faster lean gain). Observation in Experiment 7215 can be explained fairly well by this hypothesis. However, the comparisons presented in Figures 1 to 3 were gross comparisons. Statistical comparison between the responses of pigs fed to have different rates of lean mass gain was not made because of insufficient degrees of freedom. Pigs fed to have a faster rate of lean mass gain (2 and 4) had as many nuclei in the R. femoris as did pigs fed to have a slower rate of lean gain (1 and 3). Data in Table 18 do not show a significant difference in total muscle DNA for pigs of these two groups (1 vs. 4 and 2 vs. 3). Log of nuclei number:log of lean mass (Figure 1) indicated that pigs of Treatments 1 and 3 had slightly more nuclei per unit of lean mass than pigs of Treatments 2 and 4. This might or might not be a real difference.

Figure 2 revealed that the muscles of pigs fed to have a slower rate of lean mass gain had larger fiber cross-sectional area per unit of muscle lean mass than did the other groups. Therefore, muscle fibers of the slow-lean-gain pigs were shorter or had a smaller length:diameter ratio than those of the faster-lean-gain pigs. This is true only when the nuclei number and cross-sectional area of muscle fibers are in constant ratio as stated by Moss (1968b) and grossly evident in Experiment 7215 (Figure 3).

No significant effect of starter nutrition on the cellularity of L. dorsi at market weight was observed in pigs of Experiments 7107 and 7119. Also, no significant differences in nuclei number, physiological cell size or muscle fiber diameter were observed in pigs of various treatments. Although postnatal hyperplasia in the muscle fiber can be modified by energy and/or protein restrictions in the early life, the restrictions applied in the present studies were not severe enough to permanently reduce the total cell number. Evidently cellular adjustments took place after the starter period. Robinson (1969) demonstrated that active hyperplasia of porcine skeletal muscle occurs to at least 100 days after birth. Winick et al. (1968) indicated that, if previously malnourished rats were refed early enough, the normal cell number would be finally attained. This may be the case in the present studies. It is probable that compensatory hyperplasia, as well as hypertrophy, may occur during rehabilitation. The increased nitrogen retention of previously malnourished pigs during the grower period (Experiment 7119) supports the hypothesis of compensatory protein deposition. Unfortunately, no attempt was made to study the cellularity of an intact muscle during the grower

period. If it was attempted the relationships between various parameters of muscle cells might help to a better understanding of compensatory growth. Results similar to those of the present studies were also reported by Pond et al. (1969a,b) who failed to detect significant differences between cellularity of L. dorsi at market weight of controls and of pigs malnourished prenatally.

Results of Experiment 7215 indicated that protein intake did not have a significant effect on lipid concentration or number or size of adipocytes in subcutaneous adipose tissue. The lipid content per cell and lipid concentration were influenced by the rate of lean mass gain and, consequently, influenced by the confounding effect of age differences. The slower the rate of lean mass gain, (or the older the pigs get), the higher the lipid per cell or per unit of tissue weight. Similarly, the higher the lean mass, the higher the lipid concentration in adipose tissue. Again, age differences confounded the lean mass effect. No reports were found in the literature dealing with the effect of dietary protein on cellularity of adipocytes. Adipocyte hyperplasia occurs postnatally in calves (Tinyakov et al., 1968), in rats (Hirsch and Han, 1969) and in human (Bray and Gallagher, 1970). Once the adult number of adipocytes was attained, further growth or depletion of adipose tissue was accomplished only by the addition or depletion of lipid in the cells, not by alteration of cell numbers (Hirsch et al., 1966). Patterns of rat adipose tissue growth can be modified by nutrition. Undernutrition in the suckling period caused a reduction in size and number of adipocytes in epididymal fat pads of pups at 10 weeks of age (Knittle and Hirsch, 1968). Restriction in caloric intake of nursing mother caused a reduction of

size without affecting number of adipocytes in the epididymal fat pads, and the reduced cell size disappeared subsequent to refeeding (Knittle, 1972). Responses in Experiment 7215 clearly indicated that hyperplasia and hypertrophy of adipose tissue of pigs occur in postnatal life and can be modified by nutrition. The patterns of nutritional-influenced alteration may or may not be the same as seen in rats by Knittle and Hirsch (1968). This is because different patterns of hyperplasia and hypertrophy have been demonstrated in different species (DiGirolamo and Mendlinger, 1971). In addition, suckling rats in the experiment of Knittle and Hirsch (1968) were much younger physiologically when compared to postweaning pigs of Experiment 7215. In pigs, Lee et al. (1972a,b) indicated that feed restriction during a 4-week suckling period, followed by subsequent ad libitum feeding, caused reductions of number and size of adipocytes in interfascicular spaces without affecting cellularity of subcutaneous or visceral fat at 80 kg live weight. Again, these reports are not directly comparable with the results of Experiment 7215. Pigs in the reports of Lee et al. (1972a,b) were restricted fed during the sucking period and the measurements were done at 80 kg body weight or approximately 20 weeks after the feed restriction was terminated. On the other hand, pigs of Experiment 7215 were slaughtered and measurements were made right after protein restriction. The results of these experiments might have been similar if the measurements were made at a comparable condition. Compensatory hyperplasia might occur in pigs of Lee et al. (1972a,b) during the ad libitum feeding. Adipocyte hyperplasia has been shown to occur late in postnatal life, to 10 to 15 weeks of age in rats (Hirsch and Han, 1969) and to about 25 years of age in humans (Bray and Gallagher, 1970). At an

equivalent physiological maturity to the 15-week-old rat and the 25-year-old human, a pig must be much older than the 4 weeks of age. Therefore, it is reasonable to believe that some hyperplastic adjustments occurred in adipose tissue of pigs during a part of the ad libitum feeding period in the experiments of Lee et al. (1972a,b).

A Mechanism of Compensatory Response

Data in the present studies clearly demonstrated the existence of compensatory response of pigs after a period of growth retardation. However, the exact regulator(s) of the compensatory response is not easily indicated. There are several hypotheses by which the mechanisms of compensatory response can be explained. Zimmerman and Khajarern (1973) explained compensatory gain of protein, lean mass and live weight in pigs by the characteristics of normal growth curves. They considered lean growth to be "target seeking" in the sense that if lean mass was deflected from the normal growth curve by protein restriction, upon rehabilitation the lean mass would converge toward the original growth curve, and eventually, at mature weight, the lean mass of the restricted-fed pig would be the same as that of the normally-fed pig. Data from cellularity studies in Experiment 7215 tended to substantiate at least a part of this hypothesis. Assuming that the R. femoris is a representative of body lean mass, rate of lean mass gain is evidently one of the prime regulators of compensatory response. Pigs fed to have a slower lean mass gain acquired an imbalance of nuclei to muscle fiber lean mass. In agreement with the hypothesis of Cheek and Hill (1970), it was found that DNA replication was not affected as much by

protein restriction as was lean accumulation. This disrupted the normal relationship between nucleus and cytoplasm and hence implied that each nucleus was not utilized as efficiently as normal. If nuclei proliferation is rate-limiting in normal growth, then the presence of excess nuclei relative to cytoplasm in protein-restricted pigs would allow compensatory growth when adequate protein was made available. The increased grower nitrogen retention of previously-protein-restricted pigs over that of high-protein-fed pigs (Experiment 7119) suggests compensatory lean development. Unfortunately, the cellularity of muscle during the early grower period was not studied.

Compensatory growth responding to feed restriction was more evident than that seen in response to protein restriction (Experiment 7119; Zimmerman and Khajarern, 1973). Cellularity of an intact muscle was not studied in these experiments; consequently, the explanation of the mechanism for compensatory growth responding to energy restriction is purely speculative. Compensatory hyperplasia is probably a key regulator in this case. Pigs might respond similarly to rats as reported by Cheek and Hill (1970) and Hill et al. (1970), namely, the restricted-fed pigs might have had less lean, fewer nuclei and a smaller ratio of protein:DNA in muscle than the full-fed pigs. The lean mass and nuclei might subsequently be added at an accelerated rate to catch up to some genetically determined age-lean mass (DNA) relationship.

SUMMARY

1. Restriction of protein intake during the starter period caused reductions in rate and efficiency of weight gain but increased nitrogen retention per 100 grams of nitrogen consumed or absorbed in the starter period.

2. Restriction of energy intake during the starter period caused reduction of rate of weight gain but increased efficiencies of feed utilization and nitrogen retention per kilogram of body weight gain in the starter period.

3. The R. femoris of starter pigs fed a higher-protein level were heavier, had more lean (protein + water) but less fat, nuclei (DNA) and RNA and larger protein:DNA ratios than did muscle of low-protein-fed pigs. Muscle fiber diameter and RNA:DNA ratios were similar for pigs of these two groups. Muscle of faster-lean-gain pigs was heavier, had more lean and RNA, longer fiber and larger protein:DNA ratio but less fat than muscle of pigs of the other group. However, the number of nuclei, diameter of the fibers and RNA:DNA ratio were similar in muscle of pigs of these two groups.

4. Lipid content per adipose cell, concentrations of lipid and adipose cells per gram of adipose tissue of the starter pigs were not affected by level of protein intake in the period. However, pigs fed to gain lean mass at a faster rate had lower lipid per cell and per gram of adipose tissue than did slower-lean-gain pigs. The adipocyte number and all parameters relative to age were similar for pigs of these two groups.

5. Feeding the starter pigs a high protein diet caused more deposition of lean and ash but less deposition of fat in the empty body when

compared with pigs fed a lower-protein diet for the same length of time. The total empty body DNA, RNA and RNA:DNA ratio were similar for pigs of both groups; however, protein:DNA ratio was wider in the high- than in the low-protein-fed pigs. Feeding pigs to have a faster lean gain resulted in more lean but less fat deposition in the empty body than in pigs that had a slower rate of lean mass gain. Other empty body parameters observed were similar for pigs of both groups.

6. After ad libitum feeding during the subsequent growth periods, compensatory responses were observed in terms of:

Pigs that were previously restricted-fed gained weight faster and slightly more efficiently, retained more nitrogen per kilogram of body weight$^{0.75}$ and had a higher fasting level of plasma alpha-amino nitrogen in the grower period. There were no treatment differences in these parameters during the finisher period. At market weight, the previously restricted-fed pigs had slightly more water but less fat in the adjusted empty body than did the full-fed pigs.

There were no carryover effects of starter protein intake on subsequent performance or body composition of market pigs, except that previously protein-restricted pigs retained more nitrogen per kilogram of body weight$^{0.75}$ during the first part of the grower period. The reverse was true for nitrogen retention during the first part of the finisher period.

No treatment differences were observed for the L. dorsi fiber diameter or the concentrations of nucleic acids, protein and their relationships in the muscle of market pigs.

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APPENDIX

Table 20. Composition of starter diets for Experiment 7107[a]

Ingredients	Protein, % 12.0 %	14.4 %	18.0 %
Ground yellow corn	24.06	28.89	35.74
Soybean meal (48.5%)	18.62	22.31	27.92
Dried skimmilk	1.04	1.25	1.56
Dried whey	4.17	5.00	6.25
Corn starch	22.83	18.17	11.34
Dextrose	22.83	18.18	11.35
Sucrose	1.00	1.00	1.00
Stabilized lard	1.00	1.00	1.00
Solka-floc	1.07	0.97	0.84
Calcium carbonate	0.41	0.50	0.64
Dicalcium phosphate	2.06	1.83	1.47
Iodized salt	0.25	0.25	0.25
Trace mineral premix[b]	0.10	0.10	0.10
Vitamin premix[c]	0.20	0.20	0.20
Aureo S.P.-250 premix	0.25	0.25	0.25
DL methionine	0.06	0.07	0.09
Choline chloride (70%)	0.05	0.03	-

[a]Calculated analysis is presented in Table 21.

[b]Composition is presented in Table 28.

[c]Composition is presented in Table 29.

Table 21. Calculated analysis of starter diets for Experiment 7107

Items	Unit	Protein, %		
		12.0	14.4	18.0
Metabolizable energy	kcal/kg	3026.00	3026.00	3026.00
Protein	%	12.00	14.40	18.00
Calcium	%	0.70	0.70	0.70
Phosphorus	%	0.60	0.60	0.60
Vitamin A	IU/kg	3300.00	3300.00	3300.00
Vitamin D_2	IU/kg	1320.00	1320.00	1320.00
Riboflavin	mg/kg	10.93	11.37	12.01
Pantothenic acid	mg/kg	23.47	24.64	26.36
Niacin	mg/kg	40.17	40.28	40.46
Choline	mg/kg	1165.30	1171.30	1164.90
Vitamin B_{12}	µg/kg	22.00	22.00	22.00

Table 22. Composition of starter diets for Experiment 7119[a]

Ingredients	Protein, % 12.0 %	18.0 %	27.0 %
Ground yellow corn	18.31	27.55	41.33
Soybean meal (48.5%)	19.87	29.80	44.70
Dried skimmilk	0.83	1.25	1.88
Dried whey	3.33	5.00	7.50
Corn starch	25.80	15.47	-
Dextrose	25.80	15.48	-
Sucrose	1.00	1.00	1.00
Stabilized lard	1.00	1.00	1.00
Solka-floc	0.594	0.384	-
Calcium carbonate	0.40	0.61	0.92
Dicalcium phosphate	2.13	1.57	0.74
Iodized salt	0.25	0.25	0.25
Trace mineral premix[b]	0.10	0.10	0.10
Vitamin premix[c]	0.20	0.20	0.20
Aureo S.P.-250 premix	0.25	0.25	0.25
DL methionine	0.06	0.09	0.14
Choline chloride (50%)	0.02	-	-

[a]Calculated analysis is presented in Table 23.

[b]Composition is presented in Table 28.

[c]Composition is presented in Table 29.

Table 23. Calculated analysis of starter diets for Experiment 7119

Items	Unit	Protein, %		
		12.0	18.0	27.0
Metabolizable energy	kcal/kg	3040.60	3039.60	3039.90
Protein	%	12.00	18.00	27.02
Calcium	%	0.70	0.70	0.70
Phosphorus	%	0.60	0.60	0.60
Vitamin A	IU/kg	4400.00	4400.00	4400.00
Vitamin D_2	IU/kg	1101.32	1101.32	1101.32
Riboflavin	mg/kg	8.46	9.38	10.77
Pantothenic acid	mg/kg	22.93	25.64	29.62
Niacin	mg/kg	41.30	45.52	51.61
Choline	mg/kg	920.00	1132.60	1699.00
Vitamin B_{12}	µg/kg	22.00	22.00	22.00

Table 24. Composition of starter diets for Experiment 7215[a]

Ingredients	Protein, % 10.0 %	20.0 %
Ground yellow corn	19.19	38.38
Soybean meal (48.5%)	15.27	30.54
Dried skimmilk	1.04	2.08
Dried whey	4.17	8.34
Corn starch	26.29	6.94
Dextrose	26.29	6.94
Sucrose	1.00	1.00
Stabilized lard	1.00	1.00
Solka-floc	2.09	1.95
Calcium carbonate	0.18	0.70
Dicalcium phosphate	2.50	1.23
Iodized salt	0.25	0.25
Trace mineral premix[b]	0.10	0.10
Vitamin premix[c]	0.20	0.20
Aureo S.P.-250 premix	0.25	0.25
DL methionine	0.05	0.10
Choline chloride (50%)	0.13	-

[a]Calculated analysis is presented in Table 25.

[b]Composition is presented in Table 28.

[c]Composition is presented in Table 29.

Table 25. Calculated analysis of starter diets for Experiment 7215

Items	Unit	Protein, % 10.0	20.0
Metabolizable energy	kcal/kg	2990.80	2990.80
Protein	%	10.01	20.02
Calcium	%	0.70	0.70
Phosphorus	%	0.61	0.60
Vitamin A	IU/kg	4400.00	4400.00
Vitamin D_2	IU/kg	1100.00	1100.00
Riboflavin	mg/kg	8.58	10.56
Pantothenic acid	mg/kg	23.10	27.94
Niacin	mg/kg	40.79	48.18
Choline	mg/kg	1309.00	1302.00
Vitamin B_{12}	µg/kg	22.00	22.00

Table 26. Composition of grower and finisher diets for Experiments 7107 and 7119[a]

	Grower diet	Finisher diet
	Protein, %	
	16.0	12.0
Ingredients	%	%
Ground yellow corn	78.60	90.08
Soybean meal (48.5%)	18.50	8.50
Calcium carbonate	0.90	0.45
Dicalcium phosphate	1.25	0.62
Iodized salt	0.50	0.25
Trace mineral premix[b]	0.10	0.10
Vitamin premix[c]	0.10	0.05
Aurofac	0.05	0.05

[a]Calculated analysis is presented in Table 27.

[b]Composition is presented in Table 28.

[c]Composition is presented in Table 29.

Table 27. Calculated analysis of grower and finisher diets for Experiments 7107 and 7119

Items	Unit	Grower diet Protein, % 16.0	Finisher diet 12.0
Metabolizable energy	kcal/kg	2940.00	2989.00
Protein	%	16.14	12.00
Calcium	%	0.72	0.36
Phosphorus	%	0.54	0.39
Vitamin A	IU/kg	2200.00	1100.00
Vitamin D_2	IU/kg	550.00	275.00
Riboflavin	mg/kg	5.45	5.25
Pantothenic acid	mg/kg	9.19	6.36
Niacin	mg/kg	34.22	26.82
Choline	mg/kg	417.12	475.20
Vitamin B_{12}	µg/kg	11.00	5.50

Table 28. Composition of trace mineral premix

Minerals	Percent in premix	Levels in diets when added at 0.10 percent ppm
Zinc	20.00	200.00
Iron	10.00	100.00
Manganese	5.50	55.00
Copper	1.10	11.00
Cobalt	0.10	1.00
Iodine	0.15	1.50

Table 29. Amounts added to the complete diets by vitamin premix

Vitamin		Diets			
		Starter (7107)	Starter	Grower	Finisher
Vitamin A	IU/kg	3300.0	4400.0	2200.0	1100.0
Vitamin D_2	IU/kg	1320.0	1100.0	550.0	275.0
Riboflavin	mg/kg	8.8	6.6	3.3	1.6
Pantothenic acid	mg/kg	17.6	17.6	8.8	4.4
Niacin	mg/kg	39.6	33.0	16.5	8.2
Choline	mg/kg	44.0	-	-	-
Vitamin B_{12}	µg/kg	44.0	22.0	11.0	5.5
Ethoxyquin	mg/kg	-	0.4	0.2	0.1